Characterization of Clinical and Commensal *Escherichia coli* Isolates from an Integrated Turkey Operation

By
Sean Fitzgerald Altekruse
Virginia Polytechnic Institute and State University

Doctor of Philosophy in
Veterinary Medical Sciences

Francois Elvinger, Chairman

F. Willliam Pierson
Joseph D. Eifert
Daniel Karunakaran
Nammalwar Sriranganathan
External Examiner: Shaohua Zhao

October 26, 2001

Blacksburg, Virginia

Keywords: commensal, avian pathogenic *Escherichia coli*, sample size

Characterization of Clinical and Commensal *Escherichia coli* Isolates from an Integrated Turkey Operation

By
Sean Fitzgerald Altekruse
Virginia Polytechnic Institute and State University

Doctor of Philosophy in
Veterinary Medical Sciences
Fran⏩ois Elvinger, Chairman

Abstract

Pathogenic *E. coli* infections cause approximately one quarter of disease losses in commercial turkey flocks. A small subgroup of *E. coli* causes most infections. Epidemiologic studies of this disease have been hindered by a lack of reliable markers to discriminate between pathogenic and fecal *E. coli* and by the diversity of poultry strains. Reliance on antimicrobials to control *E. coli* infections has caused widespread antimicrobial resistance.

One hundred five clinical *E. coli* were obtained, and 1104 isolates were collected from fecal specimens of 20 flocks in an integrated turkey operation. Biochemical fingerprinting and antimicrobial susceptibility tests were performed on all isolates, and somatic antigen serologic testing and PCR for potential virulence genes were conducted on 299 strains including all clinical isolates and fecal isolates that had similar traits to clinical isolates. Most avian *E. coli* infections were caused by a few clonal strains that were uncommon in normal fecal flora. The potential virulence genes *iss*, K1 and *tsh* were detected more frequently among clinical than fecal isolates; however, the pattern of occurrence did not suggest that these genes were useful markers for identifying pathogenic strains.

Syndromes consistent with colibacillosis were the most commonly reported illness and principal rationale for antimicrobial therapy in sampled flocks. Most clinical *E. coli* isolates were resistant to gentamicin, sulfamethoxazole and tetracycline. Although resistance to fluoroquinolones and β-lactam antibiotics occurred less frequently, the potential for resistance to emerge to these antimicrobials was evident. A Bayesian model to estimate sample size confirmed the diversity of avian fecal *E. coli* strains.

Studies are needed to define risk factors for infection with and identify markers for avian pathogenic *E. coli* strains. These research priorities are complementary and may lead to the identification of new interventions to prevent this important infectious disease of poultry.

Acknowledgements

Many thanks to my friends, colleagues, and advisors who helped to make this project an enjoyable and rewarding experience including Jesse Arnold, Sonya Bodeis, Mike Davis, Stuart Gaines, Allan Hildesheim, Ramona Hummel, Dawn Jones, Gwendolyn Jones, Scott Komo, Kyung Lee, Sheryl Locascio, Mary Mainous, Danielle Needle, Pam Owen, Judy Rittenhouse, Debbie Saville, Mark Schiffman, Elizabeth Steele, George Terrell, Tara Tiffany, Linda Tollefson, Jocelyn Torcolini, Yanping Wang, Daniel Ward, David White, and Keying Ye.

For Martha, Will and Leah, and Mom and Dad

And out of the ground the LORD God formed every beast of the field, and every fowl of the air; and brought them unto Adam to see what he would call them: and whatsoever Adam called every living creature, that was the name thereof.

—Genesis 2:19

Contents

Tables and Figures

Tables

Figures

CHAPTER 1
Literature Review of Avian *Escherichia coli*: Laboratory Characterization, Putative Virulence Genes, Antimicrobial Susceptibility, and Statistical Methods

Background

Avian colibacillosis is a major disease of turkeys (Barnes and Gross, 1997). Surveys conducted in the U.S. indicate that *E. coli* infections occur in more than a third of production flocks, making it the leading infectious disease of commercial turkeys (Christiansen et al., 1996; Kriessel et al., 1985; Owings, 1995). Economic losses from this disease are attributed to mortality, growth depression, costs of antimicrobial therapy and carcass downgrades (Barnes and Gross, 1997). Antimicrobial agents are the cornerstone of colibacillosis control in turkey production; however, a substantial proportion of avian pathogenic *E. coli* strains have become resistant to antimicrobial drugs approved for use in poultry production (Barnes and Gross, 1997). Most colibacillosis infections are caused by a subset of pathogenic *E. coli* strains that belong to a small number of clonal lineages (White et al., 1993a; White et al., 1993b), many of which are resistant to approved antimicrobial drugs (Bass et al., 1999; Blanco et al., 1997a). Several syndromes of colibacillosis are recognized, the most important being respiratory tract infection of 3- to 12-week-old turkeys. The air sacs are generally affected early in the course of infection and systemic progression is common. Lesions of fulminant avian colibacillosis often include pericarditis, perihepatitis and septicemia.

The emergence of colibacillosis is temporally associated with the shift toward intensive turkey production in the second half of the twentieth century (Pomeroy, 1984). Consequences of modernization included intensive production (Pomeroy, 1984), genetic selection of turkeys for growth over selection for resistance to infection (Bayyari et al., 1997), and microbial adaptations such as the acquisition of virulence determinants (Reid et al., 1999). New interventions are needed to address this emerging infectious disease of poultry. The centralization of the vertically integrated industry may facilitate interventions including environmental controls (e.g., air quality and litter moisture control), and flock health programs (e.g., vaccination, biosecurity and monitoring).

Clonality

Somatic antigen serologic testing is the classic method of characterizing avian pathogenic *E. coli* strains (Pomeroy, 1984). Surveillance studies of avian pathogenic *E. coli* strains have repeatedly identified a small number of *E. coli* serogroups (e.g., O1, O2 and O78) as the etiologic agents of a large proportion of colibacillosis infections in poultry (Allan et al., 1993; Barbour et al., 1985; Blanco et al., 1998; Cloud et al., 1985; Harry, 1964; Harry and Chubb, 1964; Heller and Drabkin, 1977; Hemsley et al., 1967; Helmsley and Harry, 1965; Sojka and Carnaghan, 1961; White et al., 1993b; Whittam and Wilson, 1988). These studies of the somatic antigens of avian pathogenic *E. coli* have also shown that many strains are untypable. In addition, it is known that there is genetic diversity within (Whittam and Wilson, 1988) and similarity across (Caugant et al., 1985) *E. coli* serogroups. As a consequence a variety of other methods have been proposed to characterize pathogenic *E. coli*. These include multiple enzyme electrophoresis (White et al., 1993), Enterobacteriacea regional intergenic consensus (ERIC) PCR (Carvalho de Moura et al., 2001; Dombeck et al., 2000), pulsed-field gel electrophoresis (PFGE) (van den Bogaard et al., 2001), randomly amplified polymorphic DNA (RAPD) PCR (Chansiripornchai et al., 2001; Maurer et al., 1998b) antimicrobial resistance typing (Barbour et al., 1985; Cloud et al., 1985; David et al., 1991; Gomis et al., 2001; Heller and Smith, 1973; van den Bogaard et al., 2001), and biochemical fingerprinting (Cloud et al., 1995; Hinton et al., 1982b; Katouli et al., 1985). Somatic antigen serologic testing is the most widely recognized method for characterizing *E. coli*. Furthermore, there is no current consensus on a method of characterization to replace serologic testing. Finally, somatic antigen serologic testing and other characterization methods often provide complimentary data. Thus, it is unlikely that somatic antigen serologic testing will be entirely replaced by alternative methods for characterizing *E. coli* in the near future.

Virulence determinants/factors

The mechanisms by which avian pathogenic *E. coli* cause infection are largely unknown (Dozois and Curtiss III, 1999). Some of the factors that have been studied include factors contributing to adhesion, resistance to immunologic defense, survival in physiologic fluids, and cytotoxic effects (Dho-Moulin and Fairbrother, 1999). The specific potential virulence factors of avian pathogenic *E. coli* include F (type 1) and P fimbrial adhesins (Dho-Moulin et al., 1990; Dozios et al., 1992; Naveh et al., 1984; Pourbakhsh et al., 1997b; van den Bosch et al., 1993) and curli (Maurer et al.,

1998; Foley et al, 2000), factors conferring resistance to serum and phagocytosis (Dozios et al., 1992; Ellis et al., 1988; Pfaff-McDonough et al., 2000; Pourbakhsh et al., 1997b; Wooley et al., 1993), the aerobactin siderophores, (Dozios et al., 1992; Lafont et al., 1987; Valvano et al., 1992), *hyl*E, a hemolysin gene (Reingold et al., 1999), the *tsh* gene encoding temperature-sensitive hemagglutinin (Provence and Curtiss III, 1994) (Maurer et al., 1998a), capsular antigens (Reid and Curtiss III, 1999) and cytotoxins (Blanco et al., 1997b; Emery et al., 1992; Parreira and Yano, 1998).

Several potential virulence factors have been reported to occur at greater frequency in avian pathogenic *E. coli* strains than in commensal *E. coli* strains. These factors include aerobactin (Linggood), *iss* for increased serum survival (Pfaff-McDonough et al.) and outer membrane proteins (Chaffer et al., 1999; Kapur et al., 1992), the K1 capsular antigen (Whittam et al., 1988), and *tsh* for temperature-sensitive hemagglutinin (Dozois et al., 2000) and coligenicity (Blanco et al., 1997). In the present research, the frequency of four potential virulence genes in clinical and fecal isolates were examined: *iss*, *tsh,* K1, and *hyl*E. These potential virulence factors were selected for several reasons. We sought to replicate recent studies indicating that *iss* (Pfaff-McDonough, 2000) and *tsh* (Dozios et al., 2000) occur more frequently in clinical than fecal isolates. Interest was given to *tsh* (Brown and Curtiss III, 1996) and K1 (Vimr, 1991) because these genes may be located within unique sequences of the bacterial chromosome where other as yet unrecognized virulence genes are hypothesized to reside (Coulange et al., 2000). Furthermore, since *hyl*E was recently detected in an avian pathogenic *E. coli* strain, we therefore tested the null hypothesis that *hyl*E occurs in an equal proportion of clinical than fecal isolates (Reingold et al., 1999).

Conjugation experiments demonstrate that some of the above factors do contribute to the virulence of avian pathogenic *E. coli*. For example, when a plasmid containing genes for serum resistance and aerobactin was removed from a pathogenic *E. coli* O2 strain, virulence in inoculated birds was diminished. Virulence was restored by the reintroduction of the plasmid (Ike et al., 1992; Vidotto et al., 1991). Similarly, inoculation studies of 3-week old chickens with parent and *tsh* mutant strains of avian pathogenic *E. coli* also suggest that *tsh* contributes to the development of lesions within the air sac (Dozios et al., 2000).

Adhesion-related factors also appear to contribute to virulence. A study of FimH mutant and parent strains suggested that parent strains caused more airsacculitis than mutant strains (Arne et

al., 2000). In another study, mutants defective in the elaboration of type-1 fimbriae and flagella were 90% less adherent in cell culture assays than wild-type strains (La Ragione et al., 2000). A recent study suggests that surface polysaccharides of *E. coli* O2 bind to soluble lectin of the avian respiratory tract (Weebadda et al., 2001).

Another factor that may contribute to the pathogenesis of avian pathogenic *E. coli* infections is toxin production. Verotoxin-2 like toxin was detected in clinical isolates from chickens with swollen head syndrome (Parreira and Yano, 1998). Colicinogenicity is reported in the majority of avian pathogenic *E. coli* isolates (Emery et al., 1992; Ramirez Santoyo et al., 2001) and was associated with the highest level of pathogenicity in a study of avian pathogenic *E. coli* from Spain (Blanco et al., 1997b). The significance of cytopathic effect in the virulence of avian pathogenic *E. coli* is uncertain. In one study, nearly a quarter of 500 clinical isolates produced a cytotoxic effect (Emery et al., 1992). Other studies suggest that the ability to produce a cytopathic effect is an uncommon characteristic of avian pathogenic *E. coli* strains (Blanco et al., 1997b, Fantinatti et al., 1994). Other toxins (e.g., microcin C38) are also reported in some avian pathogenic *E. coli* strains (Leavitt et al., 1997).

Polysaccharide capsules (K antigens) are often present in *E. coli* that cause extra-intestinal disease (Jann and Jann, 1992). The K-1 capsular antigen is frequently present on certain avian pathogenic *E. coli* strains (Whittam et al., 1988). The gene cluster is sometimes referred to as a sugar island. It codes for the antigen, is approximately 20 kb in size and situated near *ser*A (Vimr, 1991). In one study the K1 capsular antigen gene was shown to enhance virulence of *E. coli* in neonatal rats (Bloch and Rode, 1996).

The mechanisms by which avian pathogenic *E. coli* strains cause invasive infections are not well understood (Dozois and Curtiss III, 1999). Many of the potential virulence factors are absent from a sizable proportion of virulent strains [e.g., hemolysin (Ramirez Santoyo et al., 2001), *iss* (Chaffer et al., 1999; Ellis et al., 1988; Pfaff-McDonough et al., 2000;), *tsh* (Dozois et al., 2000) and K1 (Whittam et al., 1988)]. It has also been shown that some of these factors (i.e., the hemolysin gene) are not sufficient to convert *E. coli* K12 into a strain that is pathogenic for poultry (Minshew et al., 1978). An observation that is difficult to reconcile with the clonality of most avian pathogenic *E. coli* strains (White et al., 1993a; White et al., 1993b) is that many potential virulence genes are carried on transmissible elements such as plasmids. These include

iss (Barondess and Beckwith, 1995; Binns et al., 1979; Chuba et al., 1989), aerobactin (Ike et al., 1992; Vidotto et al., 1991), and hemolysin (Minshew et al., 1978).

It is not known if avian pathogenic *E. coli* strains carry virulence genes on distinct chromosomal DNA segments (pathogenicity islands) comparable to those of enteropathogenic (Kaper et al., 1997) or uropathogenic *E. coli* (Guyer et al., 2001). Studies of pathogenicity islands of enteropathogenic *E. coli* strains reveal that these infections are dictated by a sequence of virulence genes (Dozois and Curtiss III, 1999). Enteropathogenic and uropathogenic *E. coli* appear to have developed into pathogens through the orderly acquisition of virulence genes over long evolutionary spans (Reid et al., 1999). Subtractive hybridization studies with an avian pathogenic *E. coli* O78 strain and *E. coli* K-12 revealed 12 unique sequences in the chromosome of the pathogenic strain containing a combined 300-400 kb that are not present in *E. coli* K-12. The insertion sites of several sequences corresponded with sites of known virulence attributes including *tsh* and pathogenicity islands PAI I and PAI II and the locus of enterocyte attachment and effacement (LEE) of enteropathogenic *E. coli* (Brown and Curtiss III, 1996). The parent strain did not exhibit properties associated with the above pathogenicity islands including attachment and effacement and erythrocyte hemolysis. It is; therefore, likely that the unique sequence present in avian pathogenic *E. coli* strain encode different proteins than those encoded by the PAI I, PAI II and LEE.

The recognition of pathogenicity islands in other pathogenic *E. coli*, evidence of the clonality of avian pathogenic *E. coli* strains, and the novel chromosomal sequences in avian strains (Brown and Curtiss III, 1996) suggest that there may be unrecognized genes for specific virulence factors in avian pathogenic *E. coli* strains. Efforts are underway in several laboratories to characterizing such sequences (Coulange et al., 2000; Dozois and Curtiss III, 1999). Sequencing of the genome of an avian pathogenic *E. coli* clone would provide information on the number and type of additional genes, beyond those found in the genome of *E. coli* K-12, including genes associated with virulence.

Barriers to flock screening

Microbiological screening is not routinely conducted to ascertain whether a flock is colonized with a strain of avian pathogenic *E. coli*. First, there is an apparent paucity of pathogenic *E. coli* in poultry environments (Barbour et al., 1985; Blanco et al., 1998; Cloud et al., 1985; Harry,

1964; Harry and Chubb, 1964; Hinton et al., 1982b; van den Bogaard et al., 2001). Furthermore, there is not a definitive strain marker for discriminating between commensal and avian pathogenic *E. coli* strains (Pfaff-McDonough et al., 2000). Finally, while assays with live birds (Pourbakhsh et al., 1997a) or embryonated eggs (Wooley et al., 2000) are available to assess the virulence of suspected avian pathogenic *E. coli* strains, the use of these procedures for routine surveillance of fecal *E. coli* from poultry flocks is cost prohibitive.

The barriers to microbiological and epidemiological studies of avian pathogenic *E. coli* infection described above have contributed to the reliance on antimicrobial therapy as the principle control for avian colibacillosis. This approach is of questionable viability given the resistance of avian pathogenic *E. coli* strains to approved antimicrobial agents (Barnes and Gross, 1997; Lambie et al., 2000; Palumbi, 2001). Microbiological and epidemiological studies of avian pathogenic *E. coli* infection are recommended to identify new interventions to control the disease problem of poultry colibacillosis.

Antimicrobial resistance

Avian pathogenic *E. coli* strains are often resistant to antimicrobials approved for poultry including tetracyclines (Bass et al., 1999; Blanco et al., 1997a; Cloud et al., 1995; Irwin et al., 1989), sulfonamides (Bass et al., 1999; Blanco et al., 1997a; Cloud et al., 1995), aminoglycosides (Allan et al., 1993; Bass et al., 1999; Blanco et al., 1997a; Dubel et al., 1982; Irwin et al., 1989) and ß-lactam antibiotics (Blanco et al., 1997a, Cloud et al., 1995). Resistance to fluoroquinolones was reported within several years of the approval of this class of drugs for use in poultry (Blanco et al., 1997a; van den Bogaard et al., 2001; White et al., 2000). There is reason for concern that genes conferring resistance to extended-spectrum β-lactams will emerge in avian pathogenic *E. coli* strains (Zhao et al., 2001) and reduce the efficacy of ceftiofur, which is currently used on a limited basis in poultry breeding flocks and hatcheries. Even though the Food and Drug Administration does not permit the use of florfenicol in poultry medicine, florfenicol resistance genes have also been detected in an avian pathogenic *E. coli* strain (Keyes et al., 2000). Evidence of resistance to florfenicol and ceftiofur are noteworthy because these drugs are candidates for use in poultry production (Salmon and Watts, 2000). Experience suggests that antimicrobial resistance genes readily emerge in the presence of the relevant selective antimicrobial pressure (Spika et al., 1987).

Colibacillosis infections caused half of all infectious disease losses in turkey production and one quarter of all disease losses in commercial turkey flocks in Minnesota in the mid 1980s (Kriessel et al., 1985). From surveys conducted in Iowa (Owings et al., 1995) and California (Christiansen et al., 1996) it is estimated that approximately one in four commercial turkey flocks experience losses due to colibacillosis. Drug costs are substantial (Christiansen et al., 1996) and the concerns about the economic implications of antimicrobial resistance and failures in the treatment of colibacillosis are justified (Lambie et al., 2000).

The insidious mechanisms by which bacteria acquire and transfer antimicrobial resistance traits underscore the need to identify alternatives to antimicrobial therapy in food animal production (Palumbi, 2001). In one study, conducted at the University of Georgia, 97 of 100 avian pathogenic *E. coli* isolates were resistant to streptomycin and sulfonamide and 87% of these multiple antimicrobial resistant strains contained a class 1 integron, intI1, which carried multiple antibiotic resistance genes (Bass et al., 1999). Multiple antimicrobial resistance traits of avian pathogenic *E. coli* have also been associated with transmissible R-plasmids (Wooley et al., 1992). When multiple resistance genes are transferred from donor to recipient bacteria, some antimicrobial resistance traits may be acquired without requisite selective pressure. Another example of insidious resistance gene transfer is the emergence of the ***cmy***2 gene. While this gene confers resistance to extended spectrum beta-lactams that are used to treat invasive salmonellosis in pediatric patients, they also confer resistance to older β-lactam drugs. Thus, even the use of a long approved drug such as penicillin can provide selective pressure for the emergence of the *cmy*2 gene (Fey et al., 2000). Unlike the transmissible antimicrobial resistance genes above, fluoroquinolone resistance is associated with the chromosomal mutations of the gyrase and topoisomerase genes (Hooper and Wolfson, 1995). Bacterial populations are thought to contain a small number of fluroquinolone resistant mutants. In the presence of selective pressure of fluoroquinolone use, resistant strains can undergo clonal expansion (Blanco et al., 1997a; White et al., 2000).

The acquisition of resistance genes is not restricted to avian pathogenic *E. coli* but is also occuring in foodborne pathogens that cause human illnesses. In the 1990s, several antimicrobial resistant foodborne bacterial pathogens emerged including a strain of *Salmonella* Typhimurium DT 104 with resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (Glynn et al., 1998), *Campylobacter jejuni* strains with resistance to ciprofloxacin

(Smith et al., 1999) and *Salmonella* with resistance to expanded spectrum β-lactam antibiotics (Fey et al., 2000). Publicity about these foodborne pathogens may undermine consumer confidence in the safety of foods of animal origin.

Dependency on antimicrobial therapy to control avian colibacillosis carries costs of medication, treatment failure, and public perception of food safety. In a recent Danish study, a higher propotion of fecal *E. coli* isolates from turkeys than other poultry species were resistant to antimicrobials (van den Bogaard et al., 2001). If the efficacy of antimicrobial therapy continues to decline as costs increase, alternative strategies for the control of colibacillosis may become attractive. The strategies might include vaccination (Abdul-Aziz et al., 1998; Peighambari and Gyles, 1998; Roland et al., 1999), competitive exclusion (La Ragione et al., 2001) and environmental controls (Poss, 1998). In the current study, we examined the antimicrobial susceptibility patterns of clinical and fecal *E. coli* strains from turkey flocks in an integrated turkey operation. We also evaluated the effect of sample size within and across flocks on estimates of geometric mean minimum inhibitory concentration (MIC). Analyses were also performed to relate flock age and antimicrobial usage to resistance (Appendix A).

Natural history of infection

The natural history of avian pathogenic *E. coli* infection has implications for control of this important infectious disease. If the natural history of avian pathogenic *E. coli* infections is consistent with the natural history of other opportunistic pathogens, then husbandry practices to prevent predisposing infections caused by other pathogens and environmental stresses are essential interventions to prevent colibacillosis outbreaks. Conversely, if avian pathogenic *E. coli* clones cause most infections (special pathogens) then efforts to exclude such clones may have merit.

The clonality of avian pathogenic *E. coli* strains is more consistent with the hypothesis that they are special pathogens (White et al., 1993a; White et al., 1993b). Furthermore, the predominant somatic antigen serogroups of clinical *E. coli* strains of poultry are reported to be more virulent than the predominant strains from fecal specimens in experimental animals (Blanco et al., 1997b). Similarly, potential virulence determinants such as *tsh* occur more frequently in clinical than in fecal strains (Dozois and Curtiss III, 1999). In that study, 91% of

avian pathogenic *E. coli* isolates that carried the *tsh* gene showed the highest level of virulence in experimental animals.

Conversely, avian pathogenic *E. coli* also exhibit features consistent with opportunistic pathogens. In particular, outbreaks of colibacillosis infection most often occur after a prior bacterial, parasitic or viral respiratory tract infection. A case-control study of mid-Atlantic turkeys showed a strong association between colibacillosis and serological evidence of prior exposure to multiple infectious agents including Newcastle disease virus, *Bordetella avium*, *Mycoplasma meleagridis*, and hemorrhagic enteritis virus (Pierson et al., 1996a). A subsequent experimental study indicated that the incidence and severity of colibacillosis increases in turkeys with sequential immune challenges to field or vaccine strains of Newcastle disease virus or *Bordetella avium* followed by hemorrhagic enteritis virus (Pierson et al., 1996b). Based on these findings the turkey industry has taken colibacillosis control measures that emphasize the evaluation of interactions between infectious agents and the careful timing of vaccinations.

The syndrome of avian colibacillosis is also consistent with an opportunistic pathogen in that it is difficult to reproduce under experimental conditions without some extenuating exposure. Experimentally, the syndrome can be reproduced by a massive aerosol exposure (Gross, 1961). Typically experimental infections are conducted with axenic birds (Bree et al, 1989). Colibacillosis can also be induced following infection by a triggering agent such as hemorrhagic enteritis virus (Larsen et al., 1985; Newberry et al, 1993; van den Hurk et al., 1994) or avian pneumovirus (Van de Zande et al., 2001) before challenge with a pathogenic *E. coli* strain. Infectious bronchitis is used as a triggering agent in broiler chickens (Smith et al., 1985). Infection with Newcastle disease virus has been shown to reduce tracheal mucous transport and bacterial clearance (Ficken et al., 1987b) and respiratory macrophage activity (Ficken et al., 1987a). A recently described high-performance experimental model for replicating the syndrome of colibacillosis requires direct inoculation of the left caudal airsac with *E. coli* (Pourbakhsh et al., 1997a).

Risk factors for infection

A small number of population-based studies have focused on attributes of flocks with and without colibacillosis (Gradel et al., 2001; Pierson et al., 1996b) or symptoms consistent with colibacillosis (Tablante et al., 1999) have been compared with those of flocks that were not

affected with colibacillosis. In a study conducted in Virginia, the antibody titers of turkeys from flocks affected by colibacillosis infection were compared with control flocks that were unaffected (Pierson et al., 1996b). Elevated titers to hemorrhagic enteritis virus, Newcastle disease virus and/or *Bordatella* were seen, either individually or collectively, in affected flocks compared to control flocks. Another study examined risk factors for early respiratory disease complex in Delmarva broiler chicken flocks (Tablante et al., 1999). Illness was associated with flock size. A study conducted in Denmark compared serologic evidence of *Salmonella* infection in broiler parent stock with and without laboratory confirmed colibacillosis. In this study colibacillosis was the dependent variable. The objective was to assess whether flocks with colibacillosis had higher rates of reaction to polyvalent *Salmonella* antisera than matched flocks without preceding colibacillosis (Gradel et al., 2001). They did not.

Experimental studies have replicated the syndrome of colibacillosis with stresses encountered in the poultry production environment. The occurrence of colibacillosis has for example, been shown to increase in the presence of environmental coliform contamination (Carlson et al., 1968). In one study, the incidence of airsacculitis doubled in turkeys exposed to high dust concentrations compared to turkeys exposed to low dust concentrations. The effect was further exacerbated by an elevation of atmospheric ammonia concentration. Pathologic lesions of exposed birds included loss of cilia from the tracheal epithelium and an increase in mucus-secreting goblet cells. Consolidation and inflammation of lung tissue were frequently observed (Anderson, 1968). In another study, *E. coli* counts were higher in the lungs, air sacs, and livers of turkeys exposed to high levels of ammonia than turkeys that were exposed to lower levels of ammonia (Nagaraja et al., 1984). High litter moisture may contribute to the risk of colibacillosis (Hayes et al., 2000) by providing a niche for ammonia producing bacteria and avian pathogenic *E. coli*. Nutritional stress (i.e., food and water deprivation for 24 to 36 hours) led to recovery of *E. coli* O78 from blood and spleen of young birds colonized with this pathogenic strain (Leitner and Heller, 1992).

Conversely, good production practices appear to protect against colibacillosis infection. The maintenance of low litter moisture and good air quality in broiler operations is important for the control of ammonia and dust in poultry houses. Strict control of these environmental factors has been shown to improve broiler performance (al Homidan et al., 1998). Similarly, appropriate levels of social stress in the production environment (Gross and Siegel, 1981) and careful

handling by human caretakers (Huff et al., 2001) appear to improve flock performance. Experimentally, an optimal level of dietary trace minerals (i.e., selinium) also reduced morbidity and mortality in chickens that were subsequently challenged with *E. coli* (Larsen et al., 1997).

Common sources of horizontal contamination with *E. coli* are other birds, hatch debris, water, and dust (Dho-Moulin and Fairbrother, 1999). In one study, environmental specimens including hatch debris, feed, dust, and litter were found to harbor *E. coli* strains belonging to serogroups O1, O2, and O78 (Nivas et al., 1977). *E. coli* strains may be introduced into the poultry environment via feed (Loken et al., 1968) or can be maintained in the darkling beetle population (*Alphitobius diaperinus*) (de las Casas et al., 1968). Although the hypothesis seems plausible (Dho Moulin and Fairbrother, 1999), we are unaware of studies that specifically demonstrate that avian pathogenic *E. coli* are vertically transmitted. It is recommended that the attributes of production flocks be characterized to facilitate the design risk factor studies of colibacillosis in turkeys (Barnes and Gross, 1997; Dho-Moulin and Fairbrother, 1999; Pomeroy, 1984) (Appendix B).

Addendum: statistical methods used to analyse data

Bootstrap

The bootstrap is a method of sampling data that provides information on the behavior of the original sample. This nonparametric statistical method is used to assess the stability of statistics (e.g., mean and confidence intervals) with repeated sampling. It is a conceptually simple but computationally demanding procedure. Personal computer software has facilitated the use of bootstrap analyses in many fields of research.

A bootstrap sample of n observations (X_1, X_2,… X_n) is drawn from a sample of original data. The bootstrap estimate of a real valued estimator (e.g., mean) is made. The original sample distribution is replaced with the bootstrap sample distribution. A probability of 1/n is given to each observation X_i. When successive iterations of resampling are performed, an estimate of the bootstrap interval for a given statistic (e.g., mean) can be obtained by Monte Carlo approximation. At least 1000 iterations are usually performed to obtain bootstrap intervals. Our rationale for performing bootstrap analyses were two-fold: First, we compared overall geometric mean MICs for fecal isolates within flocks with mean MICs within flocks when (5, 10,…45

isolates) were drawn with replacement. Secondly, we compared least square mean MIC for all 923 fecal isolates from finisher flocks and 95% confidence intervals with the bootstrap mean and interval when a fixed sample of 100 to 105 isolates was drawn in equal proportion from 5, 7, 10, and 20 finisher flocks. These analyses were performed to assess the effect of sample size on within and across flock estimates of mean MIC. The practical application of this analysis related to laboratory resources and workload.

Numeric taxonomy

We used the methods of numeric taxonomy to conduct exploratory analyses to characterize 1104 fecal *E. coli* isolates and 105 clinical isolates from turkeys. The objective was to identify isolates with similar biochemical phenotypes based on metabolism of 11 biochemicals in the PhP-Rapid *E. coli* microplate. Isolates with quite similar biochemical phenotypes (similarity coefficient greater than 0.975) were assigned named types (PhP-types). These PhP-types were used to compare clinical and fecal isolates and in a Bayesian model to predict optimal sample size for presumptive fecal *E. coli* colony selection to correctly identify all PhP-types within a flock.

Numeric taxonomy (or cluster analysis) encompasses various statistical techniques that are used to classify data into meaningful structures or taxonomies (Sneath and Sokal, 1973). Unlike statistical methods that test the significance of a priori hypotheses, the objective of cluster analyses is typically to explore data and group similar objects into clusters. A tree, or dendrogram, is often used to graphically depict results from cluster analyses. Initially, each object is assigned its own branch (similarity coefficient = 1.0). As the similarity coefficient decreases (i.e., the criterion for classifying objects as similar is relaxed) objects are joined into common branches. This process continues with the formation of larger branches that contain increasingly dissimilar elements. Ultimately, all objects join at the trunk. When the data contain objects that are similar to each other, that structure is evident as a distinct branch in the dendrogram.

When objects form their own clusters, the distance measures between objects are considered. Numerous distance measures have been developed for cluster analyses, each with their own distinct mathematical algorithms. The distance measure that was used in the analyses of biochemical phenotypes of *E. coli* isolates was Euclidean distance. This is a commonly selected distance measure. It measures the geometric distance between objects. It is computed as:

$$\text{distance}(x, y) = \{\sum_i (x_i - y_i)^2\}^{1/2}$$

with x_i and y_i being the data measurements for objects x and y.

Other distance measure include the City-block algorithm, which is similar to Euclidian distance but dampens the effect of outliers within a cluster and Chebychev distance, which tends to place objects in different groups if they differ on a measure (Sokal and Sneath, 1973).

Similar to distance measures, there are numerous algorithms for linking clusters rather than objects, referred to as linkage measures. We used UPGMA (unweighted pair-group method using arithmetic averages), which is among the most widely used linkage measures. UPGMA is less prone to chaining of clusters than the single linkage measure and is less prone than the nearest neighbor linkage measure to cause a shift to occur away from the original phenotype as membership is given to new objects and clusters (Sneath and Sokal, 1973). The UPGMA algorithm measures the distance between clusters based on average distance between objects in each cluster. Stepwise iterations begin with the joining of the most similar objects and proceed until only one cluster is left. The distance ($D_{i,j}$) between two clusters, C_i and C_j, is calculated as

$$D_{i,j} = \frac{1}{n_i + n_j} \sum_{p \in C_i} \sum_{p \in C_j} d(p,q)$$

where

n_i = $|Ci|$,

n_j = $|C_j|$,

$\sum_{p \in C_i}$ = the sum of individual measurements for C_i, and

$\sum_{p \in C_j}$ = the sum of individual measurements for C_j, for

$d(p,q)$ distances across all measurements for the two clusters.

Clusters i and j are joined to form a new cluster, ij with a node set by the similarity coefficient for i and j. The distance from cluster ij to cluster k is computed as the weighted average distance from ij to k:

$$D_{(ij),k} = (\frac{n_i}{n_i + n_j})D_{ik} + (\frac{n_j}{n_i + n_j})D_{jk}$$ UPGMA algorithm

where

$D_{(ij),k}$ = the distance between the cluster $D_{(ij)}$ and any other cluster,

$\frac{n_i}{n_i + n_j}$ = the number of objects in cluster i divided by the number of isolates in the new cluster of objects containing all objects originally in i and j,

$\frac{n_j}{n_i + n_j}$ = the number of objects in cluster j divided by the number of isolates in the new cluster of objects containing all objects originally in i and j, and

D_{ik} and D_{jk} = distances between clusters i and j and any other cluster.

The basis for these analyses was the Phene Rapid *E. coli* (PhP-RE) plate (PhPlate AB, Stockholm, Sweden). This plate contains 11 biochemicals that microbiologists at the Karolinska Institute found to provide good discrimination between strains of *E. coli*: cellobiose, lactose, rhamnose, deoxyribose, sucrose, sorbose, tagatose, D-arabitol, melbionate, Gal-lacton, and ornithine. Other biochemical panels are available with 24 or more biochemicals and provide finer discrimination. The rapid *E. coli* screening plate that was used in the present study was well suited for our purpose, providing a method for the rapid screening of large numbers of isolates that we had no reason to suspect would be similar to each other. Numeric data used in cluster analyses were the sums of microplate readings at 8, 24, and 48 hours with a 540 nm filter and bromothymol blue substrate. Similarities were calculated by unweighted pair group method with arithmetic averages using PhPWIN software (PhPlate AB, Stockholm, Sweden).

Bayesian inference

We used a statistical model with a Bayesian mode of inference to estimate optimal bacterial colony sample size, a common problem for microbiologists. If the number of distinct strains in a specimen are known then the probability of detecting them when a given number of colonies are examined can be calculated. However, the number and distribution of strains is usually not known. Our model allowed an imputation of estimates of missing data to be made in an iterative process involving prior probability statements and observed data from 20 finisher flocks.

Bayesianism (Bayes, 1763; Reprinted 1958) is a philosophy that regards knowledge as tentative and focuses on the process by which belief in a hypothesis, H is updated (i.e., the probability of H) given new evidence, E and background information, I:

$$p(H|E,I) = p(H|I) * \frac{p(E|H,I)}{p(E|I)} \qquad \text{[Bayes' rule]}$$

The term p(H|E,I) is the posterior probability. It is the probability of a hypothesis H after considering both the observed data and scientific knowledge. The term p(H|I) is the prior probability of H given I, in other words the belief in H before observed data are considered. The term p(E|H,I) gives the probability of the evidence assuming the hypothesis H and background information I is true. The last term, 1/p(E|I), is a constant that is not directly related to the hypothesis. Each probability is conditional and specifies the degree of our belief that some proposition is true (Bretthorst, 1994).

MODEL OF SAMPLE SIZE FOR MICROBIOLOGICAL STUDIES. A statistical model, with a Bayesian mode of inference, has been proposed for making probability-based estimates of sample size (Singer et al., 2000). For a particular sample size, the probability of correctly identifying all strains can be computed if the probability distribution of the actual number of strains in a flock is known. Therefore, the key point of the analysis is to estimate the distribution of the actual number of strains in a flock. The model incorporates an estimate from previous knowledge of the actual number of strains present in the object of interest, which is referred to as the prior probability statement, with actual data from a sample to give an updated estimate of the distribution, referred to as the posterior probability distribution. In our case, a Metropolis-Hastings sampling routine was used to randomly draw a sample from the distribution and the parameters of the distribution were estimated based on the sample (Tierney, 1994). This sampling routine is used to obtain estimates of random deviates of distributions for complex datasets. The objects of inference were the probabilities that a flock contained various numbers of strains.

ESTIMATING ACTUAL NUMBER OF STRAINS PER FLOCK. To diminish the effect of the starting value on the estimate of the unknown parameter (actual number of strains per flock), it is advisable to discard the initial iterations (burn-in). In our analyses the first 1000 iterations were discarded. Fifty thousand additional iterations were made and one in five iterations were used (thinning), until 10,000 iterations were obtained for analysis. Mean values from the simulations

were used as point estimates for the above parameters and 90% Bayesian intervals were placed on parameters by identifying the upper and lower 5% of simulated values.

PROBABILITY OF DETECTING STRAINS OF INTEREST BY SAMPLE SIZE. Based on posterior probability distributions, the probability of correctly identifying all strains of interest that are present in a flock can be calculated for a particular sample size (number of fecal specimens examined per flock). This probability was calculated for each draw (number of specimens collected per flock). The point estimates and 90% Bayesian interval were obtained as above. The number of specimens was varied to estimate the optimal sample size for specified sampling objectives.

CHAPTER 2
Pathogenic and Fecal *Escherichia coli* Strains from Turkeys in a Commercial Operation

Summary

Characteristics of clinical and fecal *E. coli* isolates from turkeys in an integrated operation were assessed. All of the 105 *E. coli* available isolates from clinical specimens of birds with colibacillosis were subjected to somatic antigen serologic typing and tested for potential virulence genes *hyl*E, *iss*, *tsh,* and K1. Biochemical phenotypes and antimicrobial susceptibilities of these clinical isolates were compared with those of 1,104 lactose fermenting, oxidase negative, indole producing, Gram negative rods (presumptive *E. coli*) isolated from fecal specimens from 20 turkey farms. Seventy-seven clinical isolates reacted with O-antisera of which 51 (66%) belonged to one of the following serogroups: O2, O8, O25, O78, O114 and O119. The predominant biochemical phenotype of clinical isolates contained 21 isolates including 14 isolates belonging to serogroup O78 with barely detectable β-D-glucuronidase activity. Thirty-five fecal isolates with biochemical phenotypes that matched clinical isolates in pathogenic serogroups O1, O2, O25, O78, O114 were also serogrouped and tested for potential virulence genes. Eight of these 35 (23%) isolates belonged to pathogenic serogroups O1, O25, and O78. Sixty-six of 105 (63%) clinical isolates exhibited intermediate susceptibility or resistance to gentamicin and sulfamethoxazole compared to 265 of 1104 (24%) fecal isolates ($p<0.001$). Of 167 (23%) fecal *E. coli* isolates with β-D-glucuronidase activity that exhibited this antimicrobial resistance trait, 4 (2%) belonged to pathogenic serogroups O1, O2, O25. *Iss*, K1 and *tsh* genes were detected more often among clinical than these fecal isolates ($p<0.05$). A small subgroup of pathogenic *E. coli* strains caused most colibacillosis infections in this operation and these strains existed at low concentration in normal fecal flora of healthy turkeys in intensively raised flocks. The data suggest that colibacillosis in turkey operations could be due to endogenous infections and support the hypothesis that avian pathogenic *E. coli* are specialized pathogens. Further studies are recommended of the molecular basis of pathogenesis. A review of the rationale for prophylactic injections of day old poults with potent antimicrobials such as gentamicin may also be prudent.

Introduction

Colibacillosis is a major disease of commercial turkey production (Barnes and Gross, 1997; Christiansen et al., 1996). O-antigen testing of avian pathogenic *Escherichia coli* suggests that a small number of serogroups (e.g., O1, O2, and O78) are responsible for most infections (Allan, et al., 1993; Barbour et al., 1985; Blanco et al., 1998; Cloud et al., 1985; Harry, 1964; Harry and Chubb; 1964, Heller and Drabkin, 1977, Hemsley et al., 1967; Helmsley and Harry, 1965; Sojka and Carnaghan, 1961; White et al., 1993b; Whittam and Wilson, 1988). While these serogroups are frequently recovered from internal organs of birds with colibacillosis, they are not readily detected in the environment of poultry flocks with colibacillosis (Barbour et al., 1985) or in fecal specimens from healthy birds (Blanco et al., 1998; Harry et al., 1964; Heller and Drabkin, 1977). Laboratory characterization is an important adjunct for epidemiological studies of avian pathogenic *E. coli* strains (Barnes and Gross, 1997). Many typing methods have been used to study avian pathogenic *E. coli* including somatic antigen serological typing (Allan, et al., 1993; Barbour et al., 1985; Blanco et al., 1998; Cloud et al., 1985; Harry, 1964; Harry and Chubb; 1964, Heller and Drabkin, 1977, Hemsley et al., 1967; Helmsley and Harry, 1965; Sojka and Carnaghan, 1961; White et al., 1993b; Whittam and Wilson, 1988), biochemical typing (Cloud et al., 1985), antimicrobial susceptibility testing (Barbour et al., 1985; Cloud et al., 1985; Heller and Smith, 1973) and molecular characterization (Carvalho de Moura et al., 2001; Chansiripornchai et al., 2001; Maurer et al., 1998b, van den Bogaard et al., 2001). Recently, potential virulence genes [e.g., genes for the hemolysin *hly*E (Reingold et al., 1999), increased serum survival (*iss*) (Pfaff-McDonough et al., 2000), the K1-capsular antigen (Tsukamoto, 1997) and temperature sensitive hemagglutination (*tsh*) (Maurer et al., 1998a)] have been reported to be associated with avian pathogenic *E. coli* strains (Dozois et al., 2000). In this study, we compared the biochemical phenotypes and antimicrobial susceptibility patterns of clinical and fecal isolates from an integrated turkey operation. We assessed whether these traits could be used as markers to identify pathogenic serogroups in fecal specimens. O-antigen testing was conducted on 105 clinical isolates and 194 of 1104 fecal isolates (18%) with biochemical phenotypes and antimicrobial susceptibility patterns similar to clinical isolates. The frequency of the above potential virulence genes were also determined for these isolates. We hypothesized that fecal and clinical *E. coli* isolates would share common biochemical phenotypes, antimicrobial susceptibility patterns, somatic surface antigens and potential virulence genes.

Materials and methods

Sample

During the summer of 1999, in an integrated turkey operation, six composite fecal swabs were collected from the floor of 20 finisher units containing turkeys between 10 and 14 weeks of age. Two eligible flocks were randomly selected from each of the 10 service routes of the participating integrated operation. Poults were present in 12 of the sampled houses and ranged in age from less than 1 week to 6 weeks of age (the median age was 3 weeks). Two composite fecal swabs were collected from the floor of these 12 brooder units. A total of 1104 fecal isolates were obtained, of which 923 were from finisher units and 181 were from brooder units. The fecal isolates were compared with all available clinical *E. coli* isolates from turkey flocks affected with colibacillosis in the same integrated operation between late 1997 and early 2000. These 105 clinical isolates had been maintained at the Harrisonburg Regional Laboratory of the Virginia Department of Agriculture and Consumer Services.

Isolation and identification

Fecal swabs were placed in Whirl-Pak bags containing 10 ml of buffered peptone water, transported to the laboratory at 4^{o}C, and processed within four hours. A 10^{-3} dilution of each specimen was made in peptone water and 50 µl were plated on MacConkey agar and incubated at 37^{o}C. After 18 hours of incubation, 8 lactose positive colonies were transferred to a 4-methylumbelliferyl-beta-D-glucuronide (MUG)-MacConkey agar plate (Remel Laboratories, Lenexa, KS) and incubated for 18 hours at 37^{o}C. Whenever possible, morphologically distinct colonies were selected. Colonies were examined for blue fluorescence with an ultraviolet transilluminator [peak excitation of 365nm (ultraviolet A) and a peak emission of 455nm (blue)]. Isolated colonies were transferred to nutrient agar, incubated for 18 hours at 37^{o}C and tested for indole production, oxidase activity, and gram stained. Presumptive *E. coli* were defined as indole positive, oxidase negative, gram negative, rods. A random sample of 20 presumptive *E. coli* without ß-glucuronidase activity was examined by the VITEK GNI® system (Vitek Systems, Hazelwood, MO, USA). Clinical isolates were grown on EMB agar at the time of specimen submission, tested for indole production, β-D-glucuronidase activity and Gram stain reaction. A total of 105 clinical isolates from flocks with colibacillosis were obtained from the integrated

operation between 1997 and 1999. These clinical isolates had been stored at –70°C at the regional veterinary diagnostic laboratory.

Biochemical phenotyping

Biochemical phenotypes (PhPlate, 2001) were determined for all 105 available clinical *E. coli* isolates collected from turkey flocks with colibacillosis in the integrated operation and for all 1104 lactose positive, Gram negative, indole positive, oxidase negative fecal isolates. The Phene Rapid *E. coli* (PhP-RE) plate (PhPlate, 2001) was used to assess metabolism of 11 biochemicals: cellobiose, lactose, rhamnose, deoxyribose, sucrose, sorbose, tagatose, D-arabitol, melbionate, Gal-lacton, and ornithine (PhPlate, 2001). Isolates were characterized based on the sum of microplate readings at 8, 24, and 48 hours with a 540 nm filter and bromothymol blue substrate. Similarities were calculated by unweighted pair group method with arithmetic averages using PhPWIN software (PhPlate, 2001). A 0.975 similarity coefficient was the threshold for membership within a biochemical cluster (PhP-type).

Antimicrobial susceptibility testing

The 1998 National Antimicrobial Resistance Monitoring System gram-negative Sensititre broth dilution panel (Trek Diagnostic, Westlake, OH, USA) was used to measure MICs for gentamicin (0.25 to >16 µg/ml), sulfamethoxazole (128 to >512 µg/ml) MICs and 15 other antimicrobial agents. An isolated bacterial colony was subplated from a nutrient agar plate onto a sheep blood agar plate and grown at 37°C for 18 to 24 hours. Sterile cotton tipped swabs were used to transfer colonies to tubes containing 10 ml of sterile distilled water to make a suspension with the same turbidity as a 0.5 McFarland standard. An aliquot (10 µl) of the suspension was transfered to tube containing 10 ml of sterile Mueller-Hinton broth. The inoculated broth was mixed and placed into a sterile pipette boat and 50 µl was transferred to each well of the 1999 NARMS gram-negative enteric microtiter Sensititre plate using an eight-channel pipetter. The plate was incubated at 37°C for 18 hours. MICs were recorded using a Sensitouch machine to enter the lowest concentration of antimicrobial that inhibited visible growth. Data were captured with SAMS software. Target MIC ranges were verified with *E. coli* 25922 and *Pseudomonas aeruginosa* 27853 ATCC reference strains (National Committee for Clinical Laboratory Standards, 1999).

Somatic antigen serological typing

Somatic antigen serological testing was performed on 299 *E. coli* isolates including all 105 clinical isolates and 194 fecal isolates with MICs or biochemical phenotypes similar to clinical isolates at the Pennsylvania State University Gastroenteric Disease Laboratory. Somatic antigen serological typing was conducted using a microtiter system with antibodies against 181 O-types (Orskov et al., 1977). The fecal isolates that were tested were comprised of 167 of 745 (23%) fecal *E. coli* isolates with strong β-D-glucuronidase activity on MacConkey-MUG plates. These 167 isolates were selected because they had MICs greater than 4 μg/ml for gentamicin and 256 μg/ml for sulfamethoxazole, respectively. This antimicrobial phenotype was exhibited by 63% of clinical isolates and only 24% of fecal isolates ($p<0.001$). An additional 27 fecal isolates with biochemical phenotypes matching clinical isolates in pathogenic serogroups O1, O2, O25, and O78 were also subjected to somatic antigen serological testing. A total of 35 fecal isolates with biochemical phenotypes matching these predominant clinical strains were tested including eight strains that also had the antimicrobial phenotype described above.

Potential virulence gene testing

The 299 isolates selected for somatic antigen serological typing were also examined for the presence of virulence genes at the Pennsylvania State University Gastroenteric Disease Laboratory. The presence of potential virulence genes were determined by polymerase chain reaction (PCR) using published primers for *hly*E (Reingold et al., 1999), *iss* (Pfaff-McDonough et al., 2000), K1 (Tsukamoto, 1997) and *tsh* (Maurer et al., 1998a). A 3 μl aliquot of template DNA was amplified in a reaction with 0.826 μM of primers, 18 mM of dNTPs, 4.0 mM of MgCl, 0.4 U of Taq DNA polymerase (Display Systems Biotech; Vista, CA, USA), 50 mM Tris (pH 8.3), 250 μg/ml BSA, 2% sucrose and 0.1 mM Cresol Red (Idaho Technologies; Salt Lake City, Utah, USA). Cycle conditions were optimized for each primer. Reactions were electrophoresed on 1% agarose gel and scanned by Kodak gel scanners. Positive samples were identified based on the presence of appropriate bands compared to positive controls.

Statistical analyses

Associations between attributes and source of *E. coli* isolates (i.e., clinical versus fecal) were determined by Fisher's exact test (Proc FREQ, SAS version 8e, SAS Institute; Cary, NC).

Results

A total of 1104 fecal isolates were indole positive and gram negative (presumptive *E. coli*). We obtained 923 fecal isolates from finisher flocks and 181 fecal isolates were from brooder flocks. The predominant clinical strain contained 21 biochemically similar isolates including 14 belonging to serogroup O78. Clinical isolates with this biochemical phenotype exhibited barely detectable β-D-glucuronidase activity.

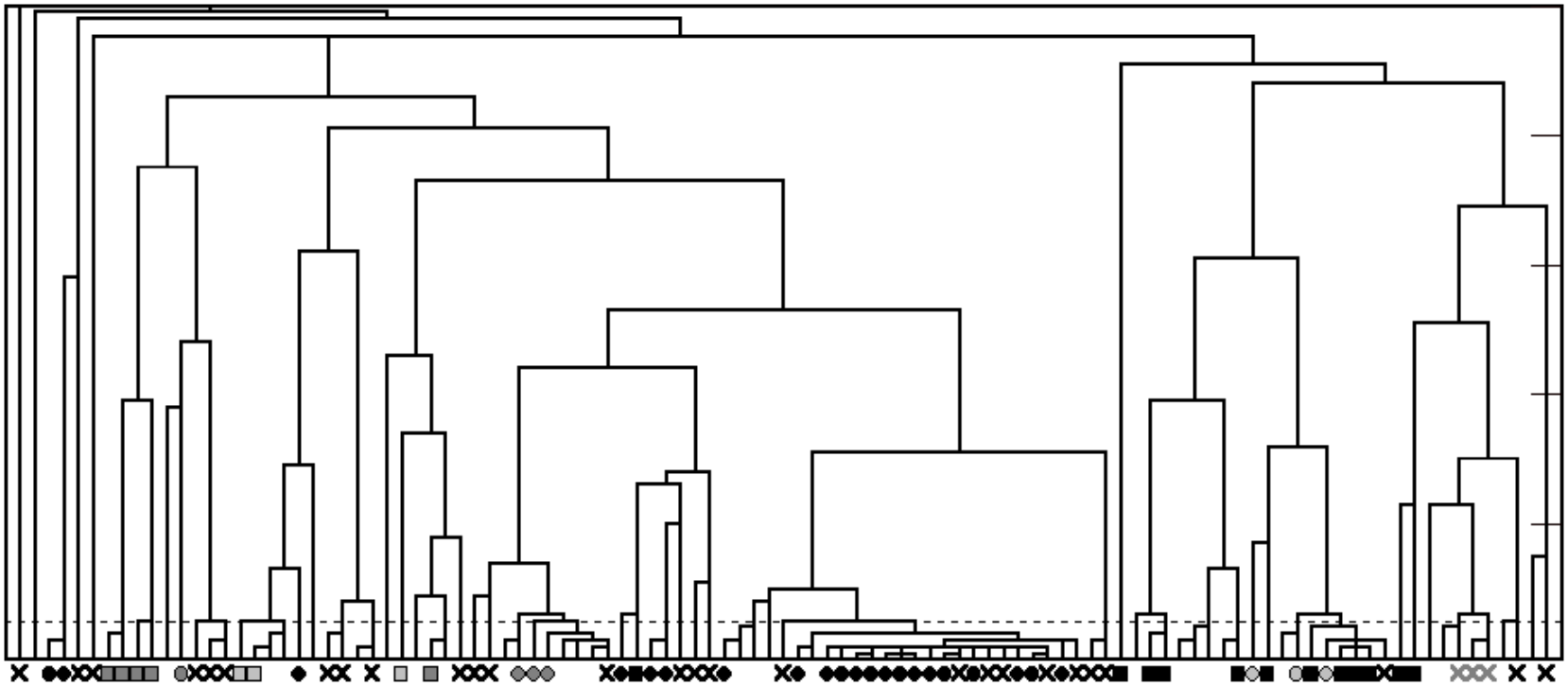
Seven-hundred-and forty-five fecal isolates (67%) exhibited readily visible β-glucuronidase activity and another 238 isolates (22%) exhibited low level β-glucuronidase activity (Appendix C) including 5 isolates in serogroup O78 ($p<0.001$). The remaining 119 isolates (11%) did not exhibit β-glucuronidase activity compared. Biochemical tests [Vitek GNI+ ®] conducted on a random sample of 20 presumptive fecal *E. coli* without detectable ß-D-glucuronidase activity confirmed that 18 were *E. coli*. One other isolate was identified as a probable *E. coli* and the other was *Klebsiella pneumoniae*.

Biochemical phenotypes

Cluster analysis of the biochemical phenotypes of clinical isolates produced a dendrogram (Figure 2.1) with one large cluster of 21 clinical isolates including 14 in serogroup O78, six that were untypable and one isolate in serogroup O20 (similarity coefficient=0.975, dotted line). Several other clusters also contained isolates with congruence between biochemical phenotype and serogroup [e.g., clusters of O2, O8, and O114]. The 10 predominant biochemical phenotypes of fecal and clinical isolates, respectively occurred in different proportions by source (Fisher's two-sided exact test, $P<0.001$). Thirty-five of 1104 (3%) fecal isolates shared common biochemical fingerprints with clinical isolates in serogroups O1, O2, O25, and O78; respectively (Table 2.1). Of these 35 fecal isolates, eight belonged to pathogenic serogroups: one to serogroup O1, two to O25 and five to O78.

Sixty-six of 105 (63%) clinical isolates exhibited MICs for gentamicin and sulfamethoxazole greater than 4 μg/ml and 256 μg/ml, respectively. In comparison, 266 of all 1104 fecal isolates (24%) exhibited this gentamicin and sulfamethoxazole resistance trait ($p<0.001$). This resistance pattern occurred more often among fecal isolates from brooder (33%) than finisher flocks (23%; $p<0.005$). Only four of 167 (2%) fecal *E. coli* with β-glucuronidase activity that exhibited the above gentamicin and sulfamethoxazole resistance trait belonged to pathogenic serogroups (i.e.,

Figure 2.1. Dendrogram of biochemical phenotypes for 105 clinical *E. coli* isolates from turkeys with colibacillosis



NOTE: Serogroup data are provided. Dotted line indicates 0.975 similarity coefficient. Black Xs (X) denote untypable isolates. Black dots (●) are isolates in serogroup O78. Black squares (■) are isolates in serogroup O2. Dark gray dots (●) denote isolates in serogroup O8. Dark gray squares (■) are isolates in serogroup O114. Gray Xs (X) are isolates in serogroup 1. Light gray dots (●) denote isolates in serogroup O25 and light gray squares (■) indicate isolates in serogroup O119.

Table 2.1. Serogroups of clinical isolates and fecal isolates with biochemical phenotypes (PhP-types) matching clinical isolates in pathogenic serogroups O1, O2, O25, O78, and O114

PhP-type[a]	No. clinical	Serogroup	No. fecal	Serogroup
A. Fecal *E. coli* with β -D-glucuronidase activity				
PhP 6	2	O1	3	O1, O9
PhP 7	5	O2, U	5	U
PhP 24	21	O20, O78, U	5	O162, U
PhP 32	2	O1, O2	5	O106, O130, U
PhP 40	1	O1	1	U
PhP 99	23	O2, O25, O78, O114, U	8	O103, U
B. Fecal isolates without detectable β-D-glucuronidase activity				
PhP 2	5	O2, O25, U	3	O25, O113
PhP 15	21	O20, O78, U	5	O78

NOTE: U = untypable.

[a]PhPWIN software clusters up to 1000 isolates. Separate comparisons were needed to compare clinical isolates to fecal isolates with and without beta-glucuronidase activity, respectively. Biochemical fingerprints and serogroups matched clinical strains for one fecal isolate in serogroup O1, two in O25 and five in O78.Gentamicin and sulfamethoxazole susceptibility

two isolates in O1 and one each in O2 and O25). Because of the low yield of pathogenic serogroups obtained when the above fecal isolates were examined, somatic antigen serological typing was not performed on fecal isolates with low-level or no β-glucuronidase activity that exhibited MICs for gentamicin and sulfamethoxazole greater than 4 μg/ml and 256 μg/ml, respectively.

Serogroups

Many isolates were untypable with standard antisera for somatic antigen serological testing including 27 of 105 (26%) clinical isolates and one "rough colony", and 71 of 194 (37%) fecal *E. coli* selected for serogrouping based on similarity to clinical isolates (Table 2.2). Among 77 (73%) clinical isolates that did react with standard antisera, 28 serogroups were identified. O78 was the predominant clinical serogroup (20% of clinical isolates), followed by O2 (11%), O114 (5%), O8 (4%), and O1, O25 and O119 (3% each). These seven serogroups combined accounted for 51 of 105 (49%) clinical isolates and 66% of the 77 clinical isolates that reacted with standard antisera. Among the 194 fecal isolates selected for somatic antigen serological typing, 123 (63%) reacted with standard antisera and 37 serogroups were identified. O19 was the

Table 2.2. Serogroups of *E. coli* from turkeys with colibacillosis and selected fecal isolates

	All isolates		Clinical isolates		Fecal isolates		
Serogroup	Count	(%)	Count	(%)	Count	(%)	p-value[a]
Untypable	98	(33)	27	(26)	71	(37)	0.07
O19	28	(9)	2	(2)	26	(13)	<0.001
O78	26	(9)	21	(20)	5	(3)	<0.001
O2	13	(4)	12	(11)	1	(1)	<0.001
O9	11	(4)	2	(2)	9	(5)	0.34
O91	10	(3)	0	(0)	10	(5)	0.02
O23	8	(3)	0	(0)	8	(4)	0.02
O8	7	(2)	4	(4)	3	(2)	0.25
O25	6	(2)	3	(3)	3	(2)	0.43
O162	6	(2)	0	(0)	6	(3)	0.09
O1	5	(2)	3	(3)	2	(1)	0.35
O15	5	(2)	0	(0)	5	(3)	0.17
O114	5	(2)	5	(5)	0	(0)	0.005
Other[b]	71	(24)	26	(25)	45	(23)	
Total	299	(100)	105	(100)	194	(100)	

NOTE: Fecal isolates with similar biochemical or antimicrobial resistance traits as clinical isolates.

[a]Fisher's two-sided exact test of proportions for clinical and fecal isolates

[b]Serogroups accounting for less than 2% of tested isolates in decreasing frequency: O98, O119, O135, O143, O159, O13, O18, O29, O39, O88, O106, O11, O22, O36, O101, O130, O153, O6, O7, O20, O53, O65, O75, O84, O86, O99, O100, O103, O105, O111, O113, O120, O131, O148, O150, O154, O171, rough.

predominant serogroup (13%), followed by O91 (5%) and O23 (8%). The three most frequent serogroups among clinical isolates (O78, O2, and O114) occurred more often among clinical than fecal isolates ($p<0.005$). Other serogroups that were isolated from clinical specimens on at least three occasions (O8, O1, O25, and O119) occurred with equal frequency among clinical and fecal isolates. Three frequently identified serogroups of fecal isolates (O19, O91, and O23) occurred more often among fecal than clinical isolates ($p<0.05$).

Virulence determinants

The *hyl*E gene was rarely detected in either clinical or fecal isolates. The *iss*, K1, and *tsh* genes occurred more often among clinical than fecal isolates selected based on antimicrobial susceptibility (Table 2.3). Three multiple gene combinations were detected more often among clinical than tested fecal isolates: 1)) *iss* and K1, 2) K1 and *tsh* and 3) *iss*, K1 and *tsh*. Among clinical isolates, K1 was seen more often in serogroups O1 and O2 [11 of 15 (73%)] than other serogroups [11 of 89 (12%)] ($p<0.001$).

Table 2.3. Frequency of virulence genes among 105 clinical *E. coli* and 167 fecal *E. coli* with MICs for gentamicin and sulfamethoxazole greater than 4 µg/ml and 256 µg/ml, respectively: increased serum survival (*iss*), temperature sensitive hemagglutination (*tsh*), hemolysin (*hyl*E), and K1-capsular antigen.

	No. clinical (%)	No. fecal (%)	p-value[a]
All tested isolates	105 (100)	167 (100)	
Individual genes in decreasing frequency			
Iss	90 (86)	92 (48)	< 0.001
tsh	62 (59)	89 (47)	0.04
K1	22 (21)	6 (4)	< 0.001
*hyl*E	4 (3)	3 (2)	0.24
Multiple genes in decreasing frequency			
iss, *tsh*	59 (56)	81 (42)	0.26
iss, K1	20 (19)	3 (1)	< 0.001
tsh, K1	12 (11)	6 (4)	0.009
iss, *tsh*, K1	12 (11)	3 (2)	< 0.001
tsh, *hly*E	4 (4)	3 (2)	0.25
iss, *hly*E	3 (3)	1 (1)	0.13
K1, *hly*E	2 (2)	0	0.54

[a]Fisher's two-sided exact test.

Discussion

A principal finding in this study was that clinical and fecal isolates exhibited different antimicrobial susceptibility patterns and biochemical phenotypes. A second finding was that pathogenic serogroups and potential virulence genes were detected more often in clinical isolates than in the comparison group of fecal isolates. These two findings did not support the hypothesis that fecal and clinical isolates were similar. An alternative hypothesis, that a few clones of *E. coli* such as strains within serogroups O1, O2, O78 cause a substantial fraction of avian colibacillosis infections , may have merit. It was noteworthy that the potential virulence genes *iss* (Pfaff-McDonough et al., 2000), K1(Tsukamoto, 1997), and *tsh* (Maurer et al., 1998a) occur significantly more often in pathogenic than fecal strains. Furthermore, avian pathogenic *E. coli* serogroups were detected in the normal fecal flora of the commercial turkey farms that we sampled but did not appear to be a major component of that flora.

Our results are consistent with other studies indicating that a small number of pathogenic *E. coli* strains (e.g., serogroups O1, O2, and O78) are responsible for a large proportion of colibacillosis infections in poultry (Allan, et al., 1993; Barbour et al., 1985; Blanco et al., 1998;

Cloud et al., 1985; Harry, 1964; Harry and Chubb; 1964, Heller and Drabkin, 1977, Hemsley et al., 1967; Helmsley and Harry, 1965; Sojka and Carnaghan, 1961; White et al., 1993; Whittam and Wilson, 1988). These were the most frequently identified serogroups in studies of turkeys colibacillosis conducted in England during the late 1950s (Sojka and Carnaghan, 1961), in Ontario during the mid 1960s (Hemsley et al., 1967), in western Canada during the mid 1980s (Allan et al., 1993), and in the midwestern United States during the 1990s (White et al., 1993b). In the present study, serogroups O8, O25, O114 and O119 were isolated from birds with colibacillosis on at least three instances each. These serogroups have also been isolated from clinical specimens of broilers with colibacillosis (Blanco et al., 1998; Hemsley et al., 1967; Sojka and Carnaghan, 1961) In this study, serogroups and biochemical phenotypes provided complementary data on *E. coli* strains, suggesting diversity within (Whittam and Wilson, 1988) and similarity across serogroups (Caugant et al., 1985). In the current study, several biochemically-distinct clusters of *E. coli* O78 were seen, including a large cluster of 14 isolates that clustered with six untypable strains. A recent study suggests that surface polysaccharides of *E. coli* O2 bind to soluble lectin of the avian respiratory tract (Weebadda et al., 2001). This interaction between surface antigens and the physiologic fluids of the avian host may explain why dissimilar avian pathogenic clones express common somatic antigens (Whittam et al., 1988). We suggest that antisera be prepared against untypable strains with biochemical phenotypes similar to the predominant strain of *E. coli* O78. The antisera may be of use to characterize pathogenic *E. coli* strains from turkeys that are currently classified untypable.

Pathogenic serogroups of *E. coli* were occasionally found in normal fecal flora of healthy turkeys in intensively raised flocks. Nonetheless, our data add to the evidence that avian pathogenic *E. coli* strains are not a major component of the environment (Barbour et al., 1985) or fecal flora of commercial poultry (Blanco et al., 1998; Cloud et al., 1985; Harry, 1964; Harry and Chubb, 1964). In a study conducted in Saudi Arabia, *E. coli* serogroups cultured from septicemic broilers were not detected in well water from the effected flock; however, both O19 and O132 were (Barbour et al., 1985). In England, pathogenic serogroups constituted a small proportion of strains isolated from the intestines of both healthy and diseased birds (Harry, 1964). In northwestern Spain, serogroups that accounted for 59% of avian colibacillosis infections accounted for only 29% of fecal isolates from healthy chickens (Blanco et al., 1998). In a study conducted in the Delmarva Peninsula none of 20 strains isolated from the yolk sacs of healthy

day-old chicks belonged to avian pathogenic serogroups (Cloud et al., 1985). Furthermore, in another study conducted in England, even when fecal strains belonged to pathogenic serogroups, many were non-pathogenic in experimental trials with day-old poultry (Harry and Chubb, 1964). It is possible that, in our study, pathogenic *E. coli* serogroups were rarely detected in fecal specimens because flocks were not colonized with these strains at the time of sampling. Alternatively, pathogenic *E. coli* strains may have been present, albeit at concentrations several logarithmic units lower than more common fecal strains.

The challenge of distinguishing pathogenic *E. coli* from other strains is heightened by the diversity of strains present in the poultry environment, which makes routine screening of poultry flocks to detect avian pathogenic *E. coli* virtually cost-prohibitive. Microbial screening might become more tenable if the reservoirs or markers of avian pathogenic *E. coli* strains were better understood. Efficient microbial screening could introduce new prospects for the control of avian pathogenic *E. coli* (e.g., reservoir identification, targeted disinfection) that would compliment currently prescribed good husbandry practices to prevent avian pathogenic *E. coli* infections (e.g., sanitation, vaccination, antimicrobial therapy).

In our study, 67% of clinical strains exhibited MICs for gentamicin and sulfamethoxazole greater than 4 μg/ml and 256 μg/ml, respectively compared to only 25% of fecal strains. This was consistent with data from a study conducted in Israel in the 1970s (Heller and Smith, 1973). In another study, gentamicin resistant *E. coli* isolates were detected more often in feces from poults than older turkeys (Dubel et al., 1982). The authors of the latter study postulated that egg dipping and injection of day old birds with gentamicin were selective pressures responsible for the elevated frequency of gentamicin resistance in fecal *E. coli* from poults. A study conducted at the University of Georgia in the late 1990s suggested a mechanism for this phenotypic trait. In that study 97 of 100 avian pathogenic *E. coli* isolates were resistant to streptomycin and sulfonamide and 87% of these multiple antimicrobial resistant strains contained a class 1 integron, intI1, which carried multiple antibiotic resistance genes (Bass et al., 1999). Since injection of day-old-birds with gentamicin was a routine practice in the operation that we studied, the association between elevated MICs for gentamicin and clinical *E. coli* strains suggests a potential source of pathogenic *E. coli*. If *E. coli* strains are transmitted, in part, through the hatchery then prophylactic use of antimicrobials there could contribute to

colonization of flocks with pathogenic strains by eliminating competitive bacterial flora that are susceptible to gentamicin, including commensal *E. coli* strains.

A longstanding poultry disease research interest has been the identification of markers for pathogenic *E. coli* strains (Brown and Curtiss III, 1996; Dho-Moulin et al. 1989; Emery et al. 1992; Maurer et al. 1998; Pfaff et al., 2000; Reingold et al., 1999; Tsukamoto, 1997). In this study, several pathogenic serogroups (e.g., O1 and O78) possessed distinct biochemical phenotypes (Cloud et al., 1985; Harry and Chubb, 1964). In a small number of instances these biochemical phenotypes were detected among fecal isolates that belonged to the corresponding serogroup. We also confirmed previous observations that clinical isolates are more likely than fecal isolates to possess the *iss* (Pfaff-McDonough et al., 2000) and *tsh* genes (Dozois et al., 2000). As previously reported, the K1 capsular antigen gene was more prevalent in specific pathogenic clones (i.e., serogroups O1 and O2) (Whittam and Wilson, 1988). Because *iss* and *tsh* were detected in nearly half of fecal isolates that we examined and K1 was present in only 21% of clinical isolates, these genes are not suitable markers for identifying avian pathogenic *E. coli* strains in fecal specimens. Further studies are recommended of the prevalence (Dozois et al., 2000; Pfaff-McDonough et al., 2000), role (Dozois et al., 2000), and location (Brown and Curtiss III, 1996, Coulange et al., 2000) of potential virulence genes in the avian pathogenic *E. coli* genome. Such studies may provide insight into the molecular basis of pathogenesis.

We used β-glucuronidase activity as a confirmatory test (Pezzlo, 1992) for presumptive *E. coli* (lactose fermenting, indole producing, oxidase negative, gram negative rods). While 89% of presumptive *E. coli* were confirmed to be *E. coli* isolates with this procedure, β-glucuronidase activity was difficult to interpret visually. Of 119 fecal isolates (11%) without β- glucuronidase activity 18 of 20 randomly selected isolates were biochemically confirmed to be *E. coli*. It was noteworthy that the predominant biochemical phenotype of serogroup O78 was only detected among fecal isolates because strains with barely detectable β-glucuronidase activity were tested for somatic surface antigens. Other *E. coli* identification protocols may have advantages related to ease and cost over the methods that we used for biochemical confirmatory testing of large numbers of presumptive *E. coli* (York et al., 2000). On the otherhand, in a study conducted in a Canadian swine operation greater than 95% of presumptive fecal *E. coli* were confirmed to be *E. coli* (Dunlop et al., 1999) when additional testing was conducted. This finding was highly consistent with our own data and raises the question of how much biochemical testing of

presumptive fecal *E. coli* isolates is needed. We suggest that the answer depends on the objective of a given study and that our procedure was more than adequate for the purpose of our analyses.

In summary, a few strains of *E. coli*, defined by biochemical phenotypes as well as somatic antigens and potential resistance genes were responsible for most laboratory confirmed colibacillosis infections in this turkey operation. These *E. coli* strains were uncommon in the normal fecal flora of healthy turkey flocks. Compared to fecal *E. coli* strains, clinical *E. coli* strains also exhibited elevated MICs to sulfamethoxazole and to the potent aminoglycoside gentamicin, which was only administed to day old poults at the hatchery. The potential for this practice to select for colonization of birds with pathogenic rather than commensal enteric bacteria merits consideration. Our results suggest that colibacillosis in turkey operations could be due to endogenous infections and support the hypothesis that avian pathogenic *E. coli* are specialized pathogens rather than opportunists affecting susceptible birds. We recommend studies to examine sources, risk factors, and the molecular basis of pathogenesis of avian pathogenic *E. coli* infections.

CHAPTER 3
Antimicrobial Susceptibilities of *Escherichia coli* Strains from a Turkey Operation

Summary

Antimicrobials are used to control avian *E. coli* infections; however, many strains are resistant to these drugs. Antimicrobial minimum inhibitory concentrations (MICs) and multiple resistance patterns were measured for 105 clinical and 1104 fecal *E. coli* strains isolated from an integrated turkey operation. More fecal than clinical isolates were resistant to ampicillin (53 versus 14 percent); however, a larger proportion of clinical than fecal isolates were resistant to ciprofloxacin (8 versus 2 percent), gentamicin (55 versus 21 percent) and sulfamethoxazole (84 versus 57 percent). Approximately 90% of isolates were resistant to tetracycline. Five multiple resistance patterns accounted for 72% of all isolates. When 15 fecal isolates were randomly selected per flock (median, 40 isolates per flock) the geometric mean MICs for ampicillin, ciprofloxacin, gentamicin and sulfamethoxazole were within a twofold dilution of the overall mean MIC in 77 out of 80 (96%) instances. When 20 or more isolates were selected per flock, the geometric mean MICs were uniformally within one dilution of the overall MICs. Bootstrap sampling was conducted to assess whether repeated samples of 100 fecal isolates drawn in equal numbers from between 5 and 20 flocks would provide results consistent with the overall geometric mean MICs for 923 fecal isolates from 20 finisher flocks. The bootstrap mean MICs were, with one exception, in the same twofold dilution range as the overall geometric mean MICs; however, 95% bootstrap intervals generally extended across two MIC dilution ranges. These data suggest that reasonable estimates of geometric mean MICs of fecal *E. coli* isolates within a flock can be made with as few as 15 isolates. Furthermore, the data suggest that a reasonable estimate of the mean MICs of fecal *E. coli* isolates across an integrated operation can be made with a sample of 105 isolates (15 isolates obtained from each of seven randomly selected flocks). Periodic sampling of fecal *E. coli* isolates using the approach proposed here assist with the selection of an appropriate antimicrobial agent and with management of the risk of antimicrobial resistance.

Introduction

Colibacillosis infections are responsible for approximately one quarter of all disease losses in turkey production (Barnes and Gross, 1997; Christiansen et al., 1996; Kriessel et al., 1985; Owings et al., 1995). The selection of an ineffective antimicrobial agent for treatment of an outbreak of avian pathogenic *E. coli* infection can cause economic losses from both prolonged illness and the cost of ineffective therapy (Barnes and Gross, 1997; Lambie et al., 2000). Antimicrobial therapy is the principal control measure for avian colibacillosis. However, pathogenic *E. coli* strains are frequently resistant to aminoglycosides, beta-lactams, sulfa drugs, and tetracyclines (Barnes and Gross, 1997; Lambie et al., 2000). An increasing proportion of avian pathogenic *E. coli* strains are resistant to fluoroquinolones (Blanco et al., 1997a; White et al., 2000). While most avian pathogenic *E. coli* are susceptible to expanded-spectrum β-lactams (Salmon and Watts, 2000) resistance to these drugs is reported among other pathogens in the family Enterobacteriacea affecting humans (Winokur et al., 2000) and food-producing animals (Bradford et al., 1999). In this study we determined the antimicrobial susceptibility patterns of clinical and fecal *E.coli* isolates from an integrated turkey operation. Sampling was conducted to assess the effect of sample size within and across flock on geometric mean MICs for ampicillin, ciprofloxacin, gentamicin and sulfamethoxazole. We hypothesized that geometric mean MIC values of presumptive fecal isolates within and across flocks could be accurately estimated with fewer than 48 presumptive fecal *E. coli* isolates per flock from each of 20 finisher flocks that were examined in this study. An optimal sample would facilitate laboratory-based surveillance of antimicrobial resistance trends in poultry production with this common indicator organism.

Materials and methods

Specimens

Six composite fecal specimens were collected from the floors of 20 finisher units with flocks between 10 and 14 weeks of age in an integrated turkey operation during the summer of 1999. Two flocks were randomly selected from each of the 10 service routes. The median placement was 11,480 birds per flock. Two additional composite fecal specimens were collected from the floor of the brooder unit when poults were present in the building. Specimens were placed in

Whirl-Pak bags containing 10 ml of buffered peptone water, transported to the laboratory at 4°C, and processed within four hours.

Isolation and identification

Fecal swabs were placed in Whirl-Pak bags containing 10 ml of buffered peptone water, transported to the laboratory at 4°C, and processed within four hours. A 10^{-3} dilution of each specimen was made in peptone water and 50 µl were plated on MacConkey agar and incubated at 37°C. After 18 hours of incubation, 8 lactose positive colonies were transferred to a 4-methylumbelliferyl-beta-D-glucuronide (MUG)-MacConkey agar plate (Remel Laboratories, Lenexa, KS) and incubated for 18 hours at 37°C. Whenever possible, morphologically distinct colonies were selected. Colonies were examined for blue fluorescence with an ultraviolet transilluminator [peak excitation of 365nm (ultraviolet A) and a peak emission of 455nm (blue)]. Isolated colonies were transferred to nutrient agar, incubated for 18 hours at 37°C and tested for indole production, oxidase activity, and gram stained. Presumptive E. coli were defined as indole positive, oxidase negative, gram negative, rods. A random sample of 20 presumptive E. coli without ß-glucuronidase activity was examined by the VITEK GNI® system (Vitek Systems, Hazelwood, MO, USA). Clinical isolates were grown on EMB agar at the time of specimen submission, tested for indole production, β-D-glucuronidase activity and Gram stain reaction. A total of 105 clinical isolates from flocks with colibacillosis were obtained from the integrated operation between 1997 and 1999. These clinical isolates had been stored at –70°C at the regional veterinary diagnostic laboratory. Only one clinical isolate was obtained from a flock in which fecal specimens were also collected.

Antimicrobial usage

At the time of the farm visit, information on all therapeutic antimicrobial usage in the flock (e.g., type of drug, rationale and time of treatment) was recorded based on interview with the field representative. The field representatives' responses were based on review of written records for each flock. The therapeutic antimicrobials in the company formulary were chlortetracycline, enrofloxacin, erythromycin, oxytetracycline, penicillin, sulfamethazine, sulfaquinoxalin, and tetracycline.

Antimicrobial susceptibility

The 1998 National Antimicrobial Resistance Monitoring System (NARMS) Sensititre MIC panel was used to measure antimicrobial resistance patterns for all 1104 fecal isolates and 105 clinical *E. coli* isolates from an integrated turkey operation (Tollefson et al., 1998). The MIC panel was a microplate-based panel with serial two-fold broth dilutions over the following ranges: amikacin (4 to 32 μg/ml), ampicillin (2 to 32 μg/ml), apramycin (2 to 32 μg/ml), amoxicillin/clavulanate potassium [augmentin, (0.5/0.25 to 32/16 μg/ml)], ceftiofur (0.5 to 16 μg/ml), ceftriaxone (0.25 to 64 μg/ml), cephalothin (1 to 32 μg/ml), chloramphenicol (4 to 32 μg/ml), ciprofloxacin (0.015 to 4 μg/ml), florfenicol (2 to 16 μg/ml), gentamicin (0.25 to 16 μg/ml), kanamycin (16 to 32 μg/ml), nalidixic acid (4 to 256 μg/ml), streptomycin (32 to 256 μg/ml), sulfamethoxazole (128 to 512 μg/ml), tetracycline (4 to 32 μg/ml), and trimethoprim/sulfamethoxazole (0.12/2.38 to 4/76 μg/ml).

National Committee for Clinical Laboratory Standards (NCCLS) interpretive criteria were used to define MIC breakpoints (NCCLS, 1999) as follows: amikacin: intermediate breakpoint 32 μg/ml, resistance breakpoint 64 μg/ml; ampicillin: intermediate breakpoint 16 μg/ml, resistance breakpoint 32 μg/ml; amoxicillin/clavulanate potassium (augmentin): intermediate breakpoint 16/8 μg/ml, resistance breakpoint 32/16 μg/ml; apramycin: no NCCLS breakpoint; ceftiofur: no NCCLS breakpoint; ceftriaxone: intermediate breakpoint 16-32 μg/ml, resistance breakpoint 64 μg/ml; cephalothin: intermediate breakpoint 16 μg/ml, resistance breakpoint 32 μg/ml; chloramphenicol: intermediate breakpoint 16 μg/ml, resistance breakpoint 32 μg/ml; ciprofloxacin: intermediate breakpoint 2 μg/ml, resistance breakpoint 4 μg/ml; florfenicol: no NCCLS breakpoint; gentamicin: intermediate breakpoint 8 μg/ml, resistance breakpoint 16 μg/ml; kanamycin: intermediate breakpoint 32 μg/ml, resistant breakpoint 64 μg/ml; nalidixic acid: no intermediate breakpoint, resistance breakpoint 32 μg/ml; streptomycin: no NCCLS breakpoint; sulfamethoxazole: no intermediate breakpoint, resistance breakpoint 512 μg/ml; tetracycline: intermediate breakpoint 8 μg/ml, resistance breakpoint 16 μg/ml; trimethoprim/ sulfamethoxazole: no intermediate breakpoint, resistance breakpoint 4/76 μg/ml. Minimum inhibitory concentrations were logarithmically transformed (log 2) for statistical analyses.

Summary of antimicrobial susceptibility data

Analyses regarding MICs were conducted on all 1104 presumptive fecal *E. coli* isolates (lactose fermenting, oxidase negative, indole producing, Gram negative rods) and 105 clinical isolates. The percentage of *E. coli* isolates in the susceptible, intermediate susceptibility, and resistance range was determined for each antimicrobial with NCCLS interpretive criteria for MIC breakpoints (Proc FREQ, SAS, Version 8; Cary, NC). Geometric mean MICs (log 2) were calculated according to origin of isolates. Deviations from normal distribution were encountered for several antimicrobials, and analysis of variance (Proc GLM, SAS, Version 8, Cary, NC) for all antimicrobials was performed on ranked data testing the 2 following hypotheses: H_0: rank of MICs for isolates from clinical specimens was equal to the rank of MICs for isolates from fecal specimens; and H_0: rank of MICs for fecal isolates from finisher flocks was equal to the rank of MICs for fecal isolates from brooder flocks. MICs greater than the high concentration in the panel were set at two times that concentration.

Multiple antimicrobial resistance patterns

Multiple antimicrobial resistance patterns were determined for five antimicrobial drugs. There are 32 mutually exclusive resistance types for these antimicrobials. Breakpoints (NCCLS, 1999) were used to define resistance (ciprofloxacin, $\geq$4 µg/ml; ampicillin, $\geq$32 µg/ml; gentamicin, $\geq$16 µg/ml; sulfamethoxazole, $\geq$512 µg/ml; and tetracycline, $\geq$16 µg/ml) and the frequency of each multiple antimicrobial resistance type was determined (Proc FREQ, SAS V8; Cary, NC). Associations between multiple resistance patterns and the source of E. coli isolates (i.e., clinical versus fecal) were determined by Fisher's exact test (Proc FREQ, SAS version 8e, SAS Institute; Cary, NC).

Screening of clinical isolates for cmy2 mediated extended-spectrum ß-lactam resistance

Two sets of published PCR primers (Zhao et al., 2001) were used to assess whether three clinical isolates with MICs for ceftriaxone greater than 16 µg/ml contained cephamycinase bla*CMY* (*cmy*2) genes, which are associated with resistance to extended-spectrum ß-lactams. The tests were conducted by Dr. Shaohua Zhoa of the Food and Drug Administration, Center for Veterinary Medicine, Division of Animal and Food Microbiology in Laurel, Maryland (Zhao et al., 2001).

Geometric mean MICs within flocks

Nine random samples of presumptive fecal *E. coli* isolates (5, 10, 15, 20, 25, 30, 35, 40 and 45 isolates per flock) were drawn with replacement from a maximum of 48 isolates in each of 20 finisher flocks. Minimum inhibitory concentrations were transformed logarithmically and means for ampicillin, ciprofloxacin, gentamicin and sulfamethoxazole were calculated for each sample (Proc MEANS, SAS V8; Cary, NC). Differences between geometric mean MICs for random samples and all isolates from a flock were calculated. One flock was excluded from analyses in which 35 or more isolates were drawn because of insufficient sample size, another was excluded from analyses of 40 or more isolates, and a third was excluded from analyses of 45 isolates.

Geometric mean MICs across flocks

Bootstrap sampling was conducted to evaluate the behavior of the geometric mean MICs when between 100 and 105 fecal isolates were drawn in equal number from across flocks in an integrated operation. Two parameters were varied: the number of isolates that were examined per flock (i.e., 5, 7, 10, and 20) and the number of flocks that were examined (i.e., 5, 10, 15 and 20 flocks in inverse order to the number of isolates examined per flock) (Resampling Stats, Add-in for Microsoft Excel v.2b; Institute for Professional Education, Arlington, VA). With 1000 iterations to sample 5, 7, 10, and 20 isolates respectively, mean MICs and 95% bootstrap intervals were determined across sampled flocks for ampicillin, ciprofloxacin, gentamicin and sulfamethoxazole. Geometric mean MICs and 95% confidence intervals for all 923 fecal isolates were also determined.

Results

Antimicrobial administration

Aminoglycosides were not used therapeutically; however, day old poults were injected with gentamicin at the hatchery. Two growth promoters were added to turkey feed at the company mill on a rotating schedule: bacitracin and virginiamycin. Six of 12 brooder flocks and 18 of 20 finisher flocks had been treated with an antimicrobial drug (Table 3.1). Antimicrobial therapy was administered to treat symptoms consistent with colibacillosis in 12 finisher flocks and two brooder flocks. Antimicrobial therapy was instituted to treat gastrointestinal symptoms in eight

Table 3.1. History of antimicrobial therapy in a sample of 12 brooder flocks and 20 finisher flocks from an integrated turkey operation

	Brooder flocks			Finisher flocks		
	No. flocks	(%)	Median age, first use (wks)	No. flocks	(%)	Median age, first use (wks)
Any drug below	6	(50)	2	18	(90)	3
Tetracyclines	2	(17)	2	11	(55)	6
Sulfa drugs	0	(0)	—	2	(10)	5
Enrofloxacin	2	(17)	2	8	(40)	5
Penicillin	5	(42)	2	10	(50)	2

NOTE: — indicates not applicable.

finisher flocks and two brooder flocks and to treat symptoms affecting miscellaneous systems in six finisher and four brooder flocks.

Characterization of fecal E. coli

Seven hundred and forty five (67%) of 1104 presumptive *E. coli* (lactose positive, indole producing, oxidase negative, gram negative rods) were confirmed to be *E. coli* based on readilyvisible ß-glucuronidase activity and another 238 isolates (22%) exhibited low level β-glucuronidase activity. The remaining 119 presumptive *E. coli* isolates (11%) did not exhibit β-glucuronidase activity. Biochemical tests [Vitek GNI+ ®] conducted on a random sample of these presumptive fecal *E. coli* without detectable ß-D-glucuronidase activity confirmed that 18 were *E. coli*. One other isolate was identified as a probable *E. coli* and the other was identified as *Klebsiella pneumoniae*.

Antimicrobial MIC distributions

Resistance to ampicillin was observed in 53% of fecal and 14% of clinical isolates ($P<0.001$) with 25% of clinical isolates showing intermediate susceptibility to ampicillin (Table 3.2). One percent of all isolates showed resistance to the extended-spectrum ß-lactam ceftriaxone. Eight percent of clinical isolates exhibited resistance to ciprofloxacin compared to two percent of fecal isolates ($P<0.001$). Nalidixic acid resistance was seen in 43% of clinical and 26% of fecal isolates ($P<0.001$). Resistance to gentamicin was observed in most clinical isolates and less than one quarter of fecal isolates ($P<0.001$). Sulfamethoxazole resistance also occurred more frequently in clinical than fecal isolates ($P<0.001$). The rate of resistance to tetracycline rounded to 90% for all isolates, regardless of source ($P=0.9$).

Table 3.2. The proportion of isolates by antimicrobial susceptibility for clinical *E. coli* and presumptive fecal *E. coli* isolates from an integrated turkey operation

Drug	*E.coli* source		Percent			Median	Mean	p-value[a]
			Susceptible	Intermediate	Resistant			
Amikacin	Clinical		97	2	1	4	4.59	0.002
	Fecal	Finisher	99	0	1	4	4.16	0.97
		Brooder	99	0	1	4	4.12	
Amoxicillin/	Clinical		85	8	8	4	4.65	0.47
Clavulinic acid	Fecal	Finisher	92	7	2	4	4.31	0.04
		Brooder	82	16	2	8	4.85	
Ampicillin	Clinical		61	25	14	4	6.0	<0.001
	Fecal	Finisher	47	2	51	64	12.9	0.003
		Brooder	35	2	63	64	19.5	
Apramycin	Clinical		—	—	—	4	4.45	<0.001
	Fecal	Finisher	—	—	—	4	3.31	0.57
		Brooder	—	—	—	4	3.25	
Ceftriaxone	Clinical		97	2	1	0.25	0.311	0.002
	Fecal	Finisher	99	0	1	0.25	0.268	0.09
		Brooder	99	1	1	0.25	0.261	
Ceftiofur	Clinical		—	—	—	0.5	0.643	<0.001
	Fecal	Finisher	—	—	—	0.5	0.516	0.88
		Brooder	—	—	—	0.5	0.526	
Cephalothin	Clinical		52	30	18	8	10.2	0.03
	Fecal	Finisher	64	24	12	8	9.54	0.60
		Brooder	54	25	20	8	9.48	
Chloramphenicol	Clinical		94	3	3	4	4.84	0.025
	Fecal	Finisher	98	1	1	4	4.35	0.87
		Brooder	98	1	1	4	4.37	
Ciprofloxacin	Clinical		90	3	8	0.015	0.066	<0.001
	Fecal	Finisher	98	1	2	0.015	0.035	0.008
		Brooder	94	4	2	0.015	0.049	
Florfenicol	Clinical		—	—	—	4	3.37	0.23
	Fecal	Finisher	—	—	—	2	3.10	0.06
		Brooder	—	—	—	4	3.30	
Gentamicin	Clinical		32	13	55	16	6.8	<0.001
	Fecal	Finisher	76	4	20	0.5	1.1	<0.001
		Brooder	62	9	29	1	1.9	
Kanamycin	Clinical		53	2	45	16	40.85	<0.001
	Fecal	Finisher	67	2	31	16	30.5	0.14
		Brooder	75	2	24	16	26.5	
Nalidixic acid	Clinical		56	1	43	4	24.0	<0.001
	Fecal	Finisher	74	2	24	4	11.1	0.005
		Brooder	65	0	35	4	16.4	
Streptomycin	Clinical		—	—	—	128	104.5	0.04
	Fecal	Finisher	—	—	—	64	81.3	0.09
		Brooder	—	—	—	64	92.8	

Drug	*E.coli* source		Percent Susceptible	Intermediate	Resistant	Median	Mean	p-value[a]
Sulfamethoxazole	Clinical		16	—	84	>512	—	<0.001
	Fecal	Finisher	42	—	58	>512	—	0.8
		Brooder	45	—	55	>512	—	
Tetracycline	Clinical		9	2	90	>32	—	0.23
	Fecal	Finisher	9	0	91	>32	—	0.01
		Brooder	14	0	86	>32	—	
Trimethoprim/	Clinical		9	2	90	0.12	0.17	<0.001
Sulfamethoxazole	Fecal	Finisher	9	0	91	0.12	0.14	0.12
		Brooder	14	0	86	0.12	0.13	

NOTES: Clinical isolates were 105 *E. coli* isolates from clinical specimens of turkeys with colibacillosis infection. Fecal isolates were 1104 lactose fermenting, indole producing, gram-negative rods (presumptive *E. coli*) isolated from fecal specimens including 923 isolates from finisher and 181 isolates from brooder flocks.

— indicates not applicable.

[a]p-value derived from analysis of variance of ranks. Two p-values are quoted for each antimicrobial. The first derives from testing H_0: rank of MICs for isolates from clinical specimens = rank of MICs for isolates from fecal specimens; the second derives from testing H_0: rank of MICs for isolates from finisher flocks = rank of MICs for isolates from brooder flocks. Farm of origin was incorporated as a source of variation. There were no NCCLS breakpoints for apramycin, ceftiofur, florfenicol or streptomycin.

Multiple antimicrobial resistance

The following five multiple antimicrobial resistance patterns each accounted for at least 5% of clinical or fecal isolates and 72% of all isolates combined (Table 3.3): 1) ampicillin, sulfamethoxazole and tetracycline; 2) ampicillin and tetracycline; 3) sulfamethoxazole and

Table 3.3. Antimicrobial resistance types accounting for at least five percent of clinical or fecal isolates from an integrated turkey operation

Multiresistant[a]	Clinical (%)	Fecal[b] (%)	p-value[c]
AST	5 (5)	235 (21)	<0.001
AT	2 (2)	199 (18)	<0.001
ST	21 (20)	152 (14)	0.08
GST	50 (48)	96 (9)	<0.001
AGST	3 (3)	102 (9)	0.03
Susceptible	7 (7)	59 (5)	0.57

[a]MICs greater than the NCCLS resistance breakpoint for the following antimicrobial agents: A=Ampicillin, C=Ciprofloxacin, G=Gentamicin, S=Sulfamethoxazole, T=Tetracycline, Susceptible=Susceptible to all five of the above antimicrobials. There were 32 possible antimicrobial resistance types for these antimicrobials, each of which was mutually exclusive.

[b]1104 presumptive fecal *E. coli* (923 isolates from finisher and 181 isolates from brooder flocks). Frequencies of antimicrobial resistance phenotypes among 745 *E. coli* isolates with ß-glucuronidase activity were similar to those for presumptive *E. coli* ($\pm$ 3 %, P>0.1)

[c]Chi-square test of equal proportions for clinical and fecal isolates

tetracycline; 4) gentamicin, sulfamethoxazole and tetracycline; and 5) ampicillin, gentamicin, sulfamethoxazole and tetracycline. No other multiple resistance type accounted for greater than 5% of isolates from either source; however, 7% of clinical and 5% fecal isolates were susceptible to all five antimicrobials.

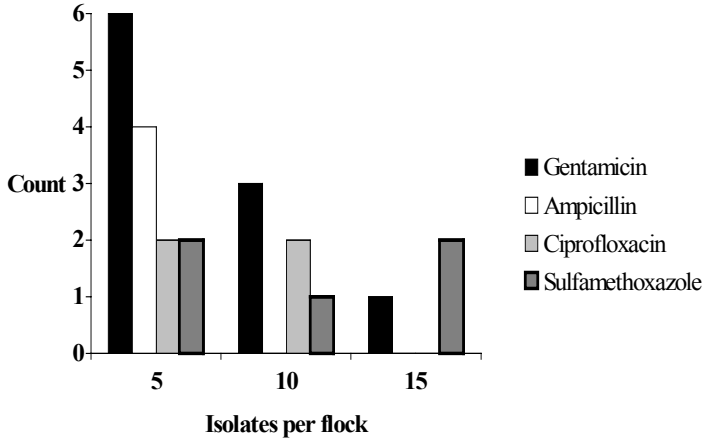
Detection of the cmy2 gene in clinical isolates

One of three clinical isolates with MIC for ceftriaxone greater than 16 µg/ml was confirmed to contain the *cmy*2 gene by polymerase chain reaction.

Mean MIC by number of isolates examined per finisher flock

Mean MICs of randomly selected isolates within flocks differed from overall means for flocks by one logarithmic unit (base 2) in 23 of 696 analyses (3%) and by two logarithmic units in three (<1%) analyses. One logarithmic unit differences were seen for all four antimicrobials (Figure 3.1). Fourteen (61%) of these instances occurred when five isolates were drawn, six (26 %) when 10 were drawn, and 3 when 15 (13%) were drawn. No such differences were observed when 20 or more isolates were drawn (test of trend, P<0.001). Two of three instances in which a two logarithmic unit difference was seen in overall and sample mean occurred when five isolates were drawn (ampicillin and gentamicin) and the other when 10 isolates were drawn (gentamicin).

Figure 3.1. Frequency of greater than 1 log unit deviation (base 2) between overall least square mean MIC and bootstrap mean MIC for ampicillin, gentamicin, ciprofloxacin and sulfamethoxazole



NOTE: Nine randomly selected presumptive fecal *E. coli* isolates (i.e.; 5, 10,… 45) were drawn from each of 20 finisher flocks. For each flock, geometric mean MICs were calculated for four antimicrobials. A sufficient sample size was available for 696 analyses. Twenty-three analyses (3%) yielded mean MICs that differed from the overall mean MIC within a flock by a two-fold dilution or greater. No deviation of this magnitude was seen when 20 or more isolates were drawn from a flock.

Mean MIC by number of flocks examined (Fixed Sample Size)

When 100 to 105 isolates were drawn from across flocks, mean MICs were within one log 2 unit of the overall geometric mean MIC for all 923 isolates (Figure 3.2) with the exception of ampicillin when isolates were drawn from five flocks (the mean MIC was greater than 16 μg/ml). With the exception of the mean MIC for sulfamethoxazole when isolates were drawn from five and 10 flocks, 95% bootstrap intervals crossed a twofold MIC dilution range.

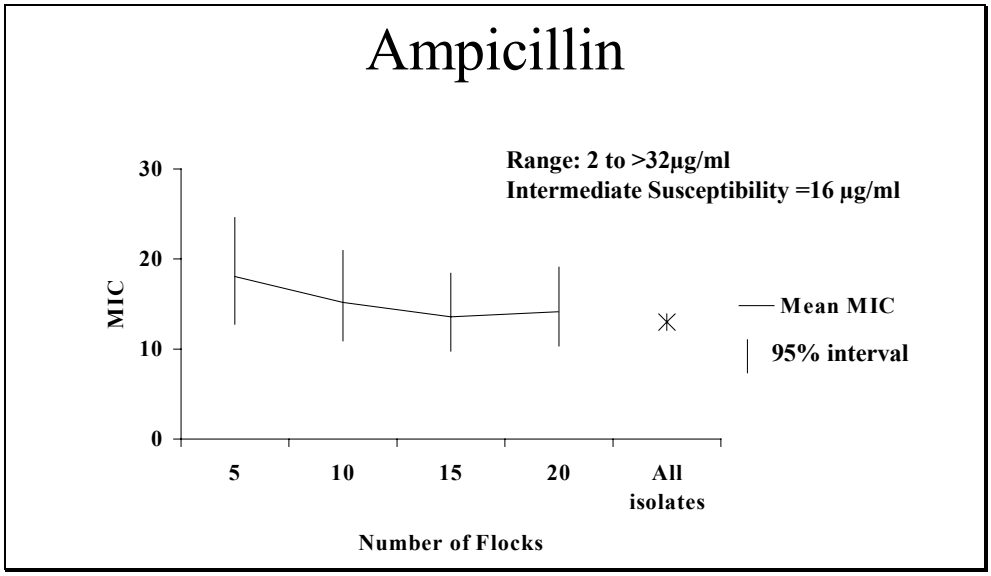
Discussion

Antimicrobial therapy was common in the flocks that were studied and was often administered to treat respiratory syndromes consistent with avian colibacillosis. Most avian pathogenic *E. coli* strains were resistant to sulfamethoxazole, tetracycline and gentamicin. Small samples of isolates provided reasonably accurate estimates of geometric mean MICs in more than 95% of instances. We could not reject the hypotheses that a sample of 15 isolates provides a reasonable estimate of the mean MICs within a flock. Similarly, a sample of 15 isolates from each of seven flocks provided a reasonable estimate of overall mean MICs across flocks in this integrated operation.

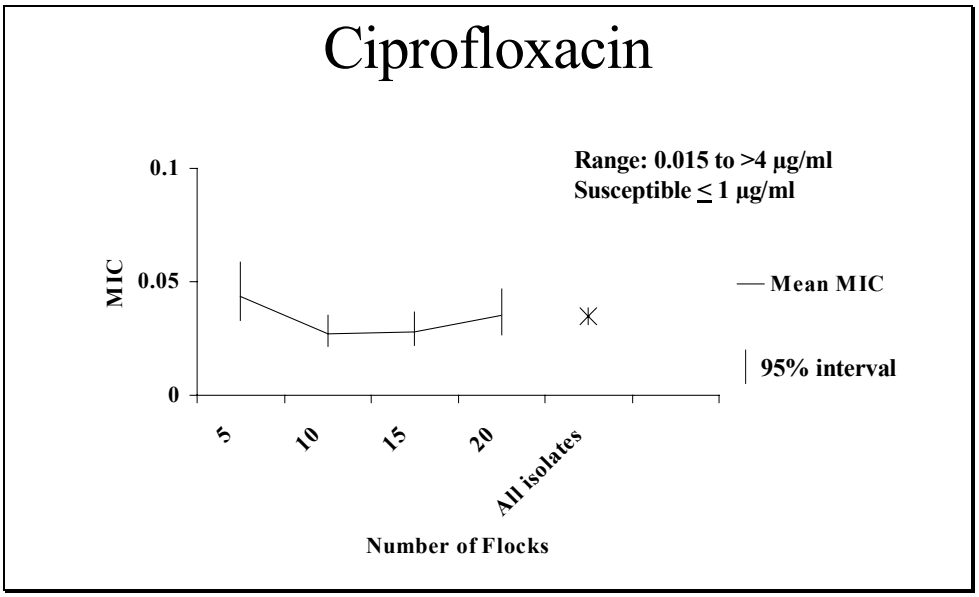
Our findings on antimicrobial usage and resistance provide little reassurance about the efficacy of antimicrobials approved for treatment of avian colibacillosis (Lambie et al., 2000). As in other studies, most avian pathogenic *E. coli* strains were resistant to tetracyclines (Blanco et al., 1997a; Bass et al., 1999; Cloud et al., 1985; Irwin et al., 1989) and sulfa drugs (Bass et al. 1999; Blanco et al., 1997a; Cloud et al., 1985). It was noteworthy that, although gentamicin was not used after poults left the hatchery, more than half of clinical isolates were resistant to this drug (Allan et al. 1993; Bass et al. 1999; Blanco et al., 1997a; Dubel et al. 1982; Irwin et al. 1989). Lower proportions of resistant isolates were seen with respect to ampicillin and fluoroquinolones; however, selective pressure could cause the frequencies of resistance to these drugs to increase. A greater proportion of fecal than clinical isolates in this study exhibited intermediate susceptibility to ampicillin, suggesting that much of the selective pressure for this antimicrobial resistance trait may occur in production flocks. Substantially higher rates of resistance to ß-lactams have been reported in avian *E. coli* strains from the Delmarva Peninsula (Cloud et al., 1985) and Northwestern Spain (Blanco et al., 1997a). In this study, three clinical isolates were also resistant to ceftriaxone and one of these isolates carried the *cmy*2 gene that confers resistance against extended-spectrum ß-lactams (Bradford et al., 1999; Winokur et al.,

Figure 3.2. Geometric mean MIC for ampicillin, ciprofloxacin, gentamicin and sulfamethoxazole for a fixed number of presumptive fecal *E. coli* drawn from finisher flocks in a turkey operation with 95% bootstrap intervals, and overall geometric mean MIC (923 isolates)

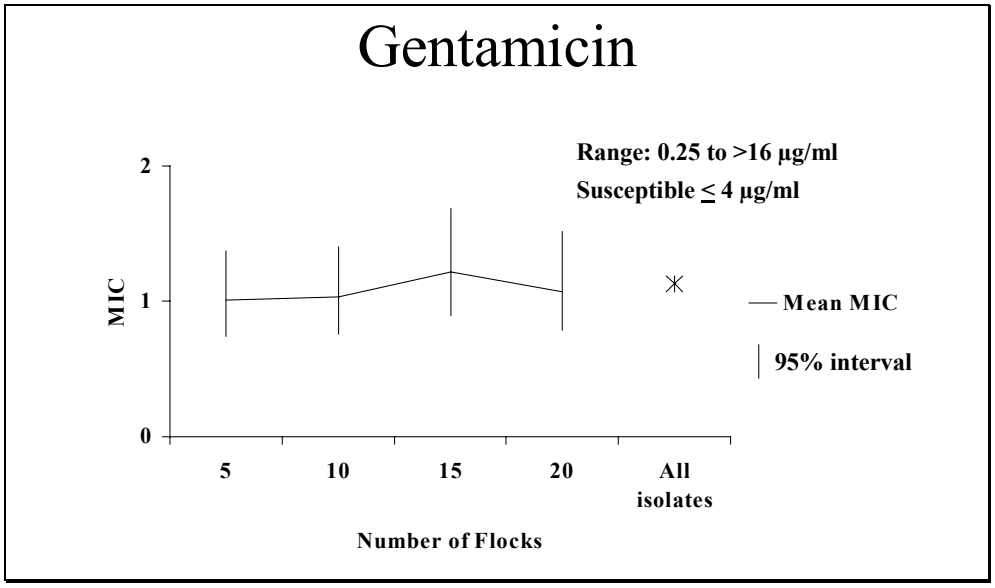
3.2a



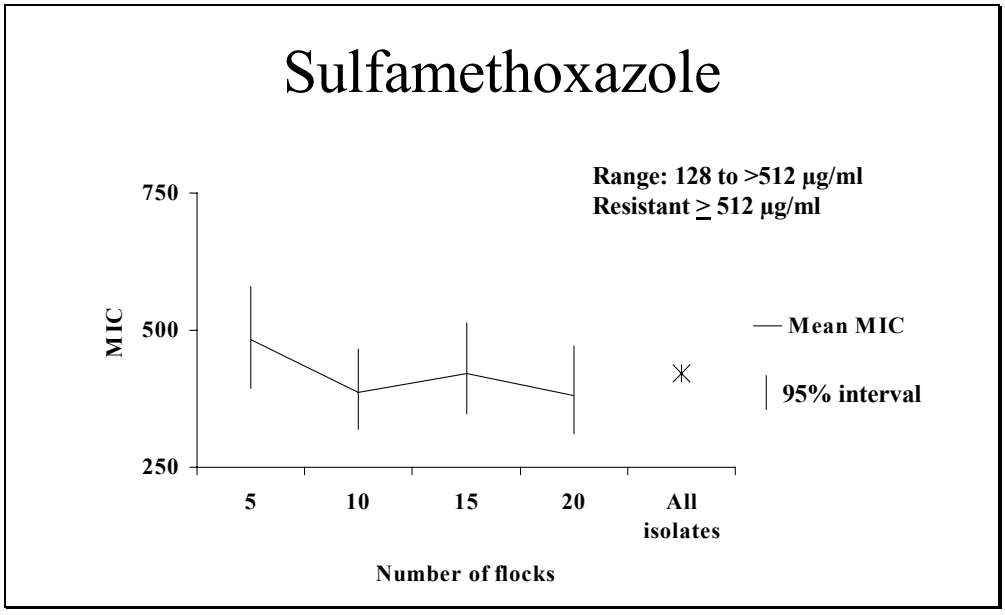
3.2b



3.2c



3.2d



NOTES: 100 isolates were drawn from 5, 10 and 20 flocks; 105 isolates from 7 flocks with 1000 iterations of sampling with replacement of 5, 7, 10 and 20 isolates from 20, 15, 10 and 5 flocks, respectively. Geometric mean MICs for all 923 presumptive fecal *E. coli* isolates from 20 finisher flocks.

2000; Zhao et al., 2001). Furthermore, while 8% of clinical isolates were resistant to ciprofloxacin, 43% were resistant to nalidixic acid. This suggests that close to half of the clinical isolates carried a mutation in the *gyr*A subunit that confers reduced susceptibility to fluoroquinolones and resistance to other quinolones (Hooper and Wolfson, 1995). One additional mutation, in the *par*C region, confers full resistance to fluoroquinolones. The emergence of fluoroquinolone resistant avian *E. coli* strains has been documented in Nortwestern Spain (Blanco et al., 1997a) and Northern Georgia (White et al., 2000). It was also noteworthy that a few multiple antimicrobial resistance patterns accounted for the vast majority of isolates. The common patterns of antimicrobial resistance may reflect the selective pressures exerted by antimicrobial usage patterns in the poultry industry (Allan et al., 1993; Bass et al., 1999; Cloud et al., 1985; Dubel et al., 1982; Irwin et al.; 1989). The findings raise concerns about the reliance on antimicrobial therapy to control avian colibacillosis. Concerns relate not only to treatment failures and marginal efficacy of antimicrobial drugs for treatment of avian colibacillosis, but also to the cost of such therapy (Christiansen et al., 1996) and the potential contribution of antimicrobial therapy to the emergence of resistant foodborne pathogens affecting humans (White et al, 2001).

Developing a framework for the judicious use of antimicrobial agents in poultry production is an industry priority (FDA, Center for Veterinary Medicine, 2001). We suggest that periodic surveillance of the MICs of fecal *E. coli* from a poultry operation could provide an important rationale for selection of a drug of choice from the available options for antimicrobial therapy. When random samples of 15 fecal isolates were drawn from within each of the 20 flocks, mean MICs were similar to the overall mean MIC value in greater than 95% of instances. It is possible that other random samples would yield different results; however, our data are consistent with findings from a study of fecal *E. coli* from Colorado dairy herds (Morley et al., 2000). In that study, mean MICs remained stable when as few as five isolates were sampled per animal or herd. Furthmore our data suggest that the mean MIC across flocks could be accurately estimated with a sample of 105 fecal *E. coli* isolates drawn in equal number from 7 randomly selected flocks in an integrated poultry operation. This type of surveillance may help poultry producers to measure (Bager, 2000; Moreno et al., 2000; Wray and Gnanou, 2000) and manage risks of antimicrobial resistance pertaining to both poultry and human health. Furthermore, if the antimicrobial drug of

choice is rotated before an elevated MIC threshold is reached, the longterm viability of approved antimicrobial agents might even be extended.

The selection of presumptive fecal *E. coli* for study was consistent with other studies that have used these bacteria as indicators for antimicrobial resistance in food animals and foods of animal origin (Wray and Gnanou, 2000). Other researchers have demonstrated that greater than 95% of presumptive fecal *E. coli* isolates are confirmed to be *E. coli* when further biochemical testing is conducted (Dunlop et al., 1999). Furthermore, individual and multiple resistant rates of confirmed and presumptive fecal *E. coli* in this study were similar.

Collection of composite fecal specimens is more efficient than the collection of specimens from individual birds. A study of finisher pigs showed that, compared to sampling of fecal specimens from individual pigs, composite fecal specimens produced unbiased and precise estimates of the prevalence of antimicrobial resistant fecal *E. coli* (Dunlop et al., 1999). Drag swabs are often used to sample the poultry environment because they are efficient and seem appropriate given the common exposure profiles of birds in a poultry flock (Mallinson et al., 1989). Furthermore, compared to collection of cloacal swabs, collection of drag swabs is less likely to cause injuries to birds. Limitations associated with the use of composite fecal swabs are; however, reported. In particular, if a swab becomes saturated, it is unlikely to pick up a bacterial strain that is clustered within one location of a house (Rolfe et al., 2000). In this study, we attempted to mitigate this limitation by collecting specimens from six equal sized areas distributed throughout each finisher room, or two such areas in brooder rooms.

In summary, most avian pathogenic *E. coli* isolates were resistant to gentamicin, sulfamethoxazole and tetracycline. Nearly half of pathogenic isolates were resistant to nalidixic acid and one quarter exhibited intermediate resistance to ampicillin. Five multiple antimicrobial resistance patterns accounted for more than 70% of clinical and fecal isolates. If the objective of sampling fecal isolates is to obtain an estimate of the resistance traits of fecal isolates within or across flocks, our data suggest that it can be accomplished with a modest allocation of laboratory resources (e.g., as few as 15 isolates per flock and seven flocks from an integrated operation). Periodic sampling of fecal *E. coli* in poultry operations may aid in the management of various risks associated with antimicrobial usage and resistance among fecal bacteria (Wray and Gnanou, 2000).

CHAPTER 4
Use of a Statistical Model to Assess Bacterial Sample Size: *Escherichia coli* Strains in Turkey Flocks

Summary

A Bayesian model was used to analyze data on fecal *E. coli* isolates from 20 turkey finisher flocks. The iterative model incorporated assumptions and observed data to estimate probabilities of correctly identifying fecal *E. coli* strains of interest in a flock by the number of bacterial colonies examined.

Three analyses were conducted. Bacterial colony sample size was nested within the number of fecal specimens collected since eight colonies were isolated from each specimen. The first analysys assessed the sensitivity of the model with various prior probability statements. This analysis was conducted with a discrete dataset of 566 of 632 *E. coli* isolates with ß-glucuronidase activity that had biochemical phenotypes that were observed at least twice (similarity coefficient >0.975). The second analysis assessed the effect of sample diversity on the probability of identifying all strains. One dataset was restricted to 833 presumptive fecal *E. coli* isolates with reoccurring biochemical phenotypes, the other was comprised of all 923 presumptive fecal *E. coli* isolates including 64 unique strains. A third analysis was conducted to estimate the optimal number of colonies to examine to correctly identify five common strains if present in a flock.

Although prior probability statements in the first analysis were different from one another, they were all relatively consistent with the observed data and did not markedly influence estimated sample size. In the second analysis, it was estimated that at least 19 fecal specimens (152 colonies) would be needed to identify all *E. coli* strains with 95% probability. In the last analysis, it was estimated that the five common strains could be identified, if present, with two fecal specimens (16 isolates) per flock. The results suggest that fecal *E. coli* isolates from turkey flocks are diverse and that a few predominant strains are present across flocks. The model oindicates the extent to which optimal sample size varies depending on the objective. This model permits the update of sample size estimates when unknown or variable numbers of strains are anticipated. It may be particularly valuable for when ongoing microbiological studies are anticipated of food specimens or in animal populations.

Introduction

E. coli is a common indicator organism. It is widely used in the poultry industry to assess fecal contamination of poultry products (Bailey et al., 2000). There is increasing concern about the decline in antimicrobial susceptibility of *E. coli* strains that cause infections of poultry (Lambie et al., 2000; Barnes and Gross, 1997). There is also interest in the antimicrobial susceptibility of isolates from poultry carcasses (Turtura et al., 1990) and poultry feces (van den Bogaard et al., 2001). *E. coli* isolates are used as a proxy measure for the source of fecal contamination in river systems (Parveen et al., 2001) and the human environment (Dombeck et al., 2000)]. Despite the significance of *E. coli* strains to the poultry industry unanswered questions remain including questions on the amount of diversity to be expected among fecal *E. coli* isolates within poultry and other livestock populations (Hinton et al., 1982b; van den Bogaard 2001; Katouli et al., 1995) and on the similarity of strains present in multiple flocks across integrated poultry operations (Hinton et al., 1982b; van den Bogaard et al., 2001). Microbiologists face a familiar challenge when allocating resources to address such questions (Kumar et al., 1972; Caldwell et al. 1994). When excessive sampling is conducted, valuable and limited laboratory resources are wasted (Berkelman et al., 1994). If insufficient sampling is conducted, biased inferences are possible (Singer et al., 2000).

Singer et al. (2000) described a statistical model to estimate the number of *E. coli* colonies to examine to correctly identify all phenotypes present in avian cellulitis lesions. A Bayesian approach was used that combined observed data and input in the form of a prior probability statement or specification. An iterative simulation was used to generate a posterior distribution. Following this approach, we proposed a simplified statistical model to estimate the optimal number of fecal specimens to collect from turkey finisher flocks for two very different sampling objectives: 1) correctly identifying each *E. coli* strain in a flock and 2) detecting widely dispersed strains, if present in a flock. It was hypothesized for the purpose of running these models that the collection of six specimens per flock and eight presumptive *E. coli* colonies per specimen was an adequate sample size for both analyses.

Methods

During the summer of 1999, in an integrated turkey operation, six composite fecal swabs were collected from the finisher unit floor of 20 turkey flocks with hens between 10 and 14 weeks of

age. Two flocks were randomly selected from each of the 10 service routes. Specimens were placed in Whirl-Pak bags containing 10 ml of buffered peptone water transported at 4^{o}C, and processed at the laboratory within four hours.

Isolation and identification

A 10^{-3} dilution of each specimen was made in peptone water and 50 µl were plated on MacConkey agar and incubated at 37^{o}C. After 18 hours of incubation, 8 lactose positive colonies were transferred to a 4-methylumbelliferyl-beta-D-glucuronide (MUG)-MacConkey agar plate (Remel Laboratories, Lenexa, KS) and incubated for 18 hours at 37^{o}C. Whenever possible, morphologically distinct colonies were selected. Colonies were examined for blue fluorescence with an ultraviolet transilluminator [peak excitation of 365nm (ultraviolet A) and a peak emission of 455nm (blue)]. Isolated colonies were transferred to nutrient agar, incubated for 18 hours at 37^{o}C and tested for indole production, oxidase activity, and gram stained. Presumptive E. coli were defined as indole positive, oxidase negative, gram negative, rods. A random sample of 20 presumptive E. coli without ß-glucuronidase activity was examined by the VITEK GNI® system (Vitek Systems, Hazelwood, MO, USA).

Subtyping

Escherichia coli strains were defined by their biochemical phenotypes. The Rapid *E. coli* plate (PhP-RE, PhPlate AB, Stockholm, Sweden) was used to assess the ability of strains to metabolize 11 biochemicals: cellobiose, lactose, rhamnose, deoxyribose, sucrose, sorbose, tagatose, D-arabitol, melbionate, Gal-lacton, and ornithine (PhPlate, 2001). Isolates were characterized based on the sum of microplate readings of light absorbance at 540 nm in bromothymol blue dye substrate at 8, 24 and 48 hours. Calculations of similarities were conducted by unweighted pair group method with arithmetic averages using PhPWIN software (PhPlate AB, Stockholm, Sweden). A 0.975 similarity coefficient was used as the minimum threshold to place isolates into common biochemical phenotypes (PhP-types).

Statistical analysis

A statistical model with a Bayesian mode of inference has been proposed for the purpose of making probability-based estimates of bacterial colony sample size for microbiological studies

(Singer et al., 2000) which we adapted and streamlined using a different simulation scheme to do the analysis. The execution in computation was expected to take less CPU time.

For a particular sample size, the probability of correctly identifying all strains can be computed if the probability distribution of the actual number of strains in a flock is known. Therefore, the key point of the analysis is to estimate the distribution of the actual number of strains in a flock. The Bayesian model incorporates an estimate from previous knowledge of the actual number of strains present in the object of interest, which is referred to as the prior probability statement, with data from an actual sample to give an updated estimate of the distribution, referred to as the posterior probability distribution. A Metropolis-Hastings sampling routine was used to randomly draw a sample from the distribution and the parameters of the distribution were estimated based on the sample. Here, the unknown parameters were the true probabilities that a flock contained various numbers of strains.

For each analysis the first 1000 iterations were discarded to diminish the effect of the starting value (burn-in). Fifty thousand additional iterations were made and one in five iterations were used (thinning), until 10,000 iterations were obtained for analysis. Mean values from the simulations were used as point estimates for the above parameters and 90% Bayesian intervals were placed on parameters by identifying the upper and lower 5% of simulated values.

Based on posterior probability distributions, the probability of correctly identifying all strains of interest that were present in a flock can be calculated for a particular sample size (number of fecal specimens examined per flock). This probability was calculated for each draw from the posterior probability distribution of the actual number of strains and the point estimates as well as the 90% Bayesian interval were obtained as above. The number of specimens was varied to estimate the optimal sample size for specified sampling objectives.

FIRST ANALYSIS: EFFECT OF PRIOR PROBABILITY STATEMENT. This analyses was restricted to 565 fecal *E. coli* isolates with ß-glucuronidase activity and biochemical phenotypes that were observed at least twice in finisher flocks (Table 4.1). This dataset was used to assess the extent to which posterior distributions were influenced by prior probability statements. Prior probability statements assumed between 9 and 24 strains were present at equal concentration in a given flock (16 possibilities). Dirichlet distributions were used. To assess the influence of the prior probability statement, we used various Dirichlet distributions and weights of the prior statements.

Table 4.1. Data used in three analyses with a Bayesian model to estimate probability of correctly identifying *E. coli* strains with various colony sample sizes

Analysis	One	Two	Three
Model input of interest	Prior assumptions	Diversity of strains	Five common strains
Database	Confirmed *E. coli*[a]	Presumptive *E. coli*	Widely distributed strains
No. matched isolates	566	859	225
No. PhP-types	94	99	5
Unique strains	66[b]	64[c]	0
Total isolates	632	923	225

NOTE: Analysis One examined the effect of prior probability statements on posterior distributions with a dataset of 566 *E. coli* strains with ß-glucuronidase activity. Analysis Two examined the effect of strain diversity on posterior distributions with 859 presumptive fecal *E. coli* isolates and 923 presumptive fecal *E. coli* isolates (additional 64 unique strains). Analysis Three was performed to estimate the optimal sample size to correctly identify five widely distributed strains if present in a flock. Strains were defined by PhP-types with similarity coefficients $\geq$0.975.

[a]*E. coli* with ß-glucuronidase activity

[b]Unique biochemical phenotypes were excluded from analysis.

[c]Unique biochemical phenotypes were excluded in one of two models.

The weight of the prior was increased or decreased by changing the cumulative values of the prior probability distribution. We used "prior sample size" (Singer et al., 2000), which was defined as the sum of the parameters of the prior Dirichlet distribution, to measure the weight of the prior distribution. Simulations were conducted with three prior probability statements to assess their effect on posterior distributions. The first statement specified a uniform prior Dirichlet (1/16, 1/16,…, 1/16). The cumulative value of this prior probability distribution summed to one, and was therefore comparable in weight to observed data for one flock. The two additional prior probability statements both had prior sample sizes of 32: a uniform prior probability Dirichlet (2, 2,…, 2) and a symmetrically distributed prior probability with modes at 16 and 17 isolates per flock: Dirichlet (0.32, 0.32, 0.64, 1.28, 1.92, 2.56, 3.52, 5.44, 5.44, 3.52, 2.56, 1.92, 1.28, 0.64, 0.32, 0.32). Observed data were input into the model as counts of the number of strains identified in each of the 20 flocks (Table 4.2).

SECOND ANALYSIS: EFFECT OF STRAIN DIVERSITY ON MODEL. Analyses were conducted to estimate the probability of correctly identifying all presumptive fecal *E. coli* strains in a flock as sample size increased; one with 833 isolates with biochemical phenotypes found at least twice in enrolled flocks and a second with all 923 isolates. The actual range of the number of strains was assumed to be [10, 35] and the prior used is a uniform, Dirichlet (0.867, 0.867) with a "prior sample size" equal to 22.5.

Table 4.2. Distribution of strains in 20 finisher flocks (observed data) for three Bayesian analyses

Objective of analysis and strains in model																			
One: Prior probability statement	566 presumptive *E. coli* isolates with ß-glucuronidase activity																		
Number of strains per flock	9	10	11	12	13	14	15	16	17	18	19	20	21						
Number of flocks	2	1	0	2	2	3	0	2	4	1	2	0	1						
Two: Strain diversity	833 presumptive *E. coli* isolates [a]																		
Number of strains per flock	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
Number of flocks	1	1	0	2	4	0	5	1	1	1	2	0	2	0	0	0	0	0	
	All 923 presumptive *E . coli* isolates [a]																		
Number of strains per flock	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
Number of flocks	1	0	0	0	2	0	0	0	1	1	4	3	1	2	2	1	1	1	
Three: Widely distributed strains	225 widely distributed *E . coli* isolates																		
Number of strains per flock	1	2	3	4	5														
Number of flocks	2	5	11	2	0														

[a]Matching isolates were defined by biochemical phenotypes (PhP-types) with similarity coefficients greater than 0.975. In no instances were greater numbers of strains than those listed observed per flock.

THIRD ANALYSIS: SAMPLE SIZE TO IDENTIFY COMMON STRAINS. A third analysis was performed to estimate probabilities of correctly identifying five widely distributed biochemical phenotypes of *E. coli* (Table 4.2), if present in a flock, when various colony sample sizes were selected. The effect of two prior probability statements on estimated optimal sample size were evaluated, both with a prior sample size of five. The first prior probability statement had a uniform distribution (i.e., Dirichlet (1, 1, 1, 1, 1) of between one and five widely distributed biochemical fingerprints of *E. coli* per flock and the second was symmetrically distributed with a peak at the midrange of three strains per flock: Dirichlet (0.25, 1, 2.5, 1, 0.25).

Model development

Suppose the range of the number of strains actually present on a farm is from 1 to k. Let y_i denote the number of farms on which i strains were observed and θ_i denote the probability that a farm actually contains i strains. Denote the vector $(y_1, y_2,\ldots, y_k)$ and vector $(\theta_1, \theta_2,\ldots,\theta_k)$ as Y and Θ, respectively. For a given sample size, let $p_{j|i}$ denote the probability of observing j strains from a farm which actually contains i strains and p_j denote the probability of observing j strains from a farm. With the use of basic principles of probability, we can compute p_j as in (A.1):

$$p_j = \sum_{i=1}^{k} \theta_i * p_{j|i} \quad \text{(A.1)}$$

There are n farms and the probability that we observe j strains from each farm is p_j, where j=1, 2,.,k. Thus, by definition, Y vector follows a Multinomial distribution, as in (A.2):

$$Y|\Theta=(y_1, y_2,\ldots, y_k)\sim\text{Multinomial}(n; p_1, p_2,\ldots, p_k) \quad \text{(A.2)}$$

Denote the prior distribution by $\pi(\Theta)$. It is natural to use Dirichlet distribution in the model since it is a vector of probabilities. So,

$$\pi(\Theta)\sim \text{Dirichlet}(a_1, a_2,\ldots, a_k) \quad \text{(A.3)}$$

(A.1), (A.2) and (A.3) give the posterior distribution as in (A.4).

$$\pi(\Theta|Y)(\theta_1^{a_1-1}\theta_2^{a_2-1}\ldots\theta_k^{a_k-1}p_1^{y_1}p_2^{y_3}\ldots p_k^{y_k}$$

$$\propto \theta_1^{a_1-1}\theta_2^{a_2-1}\ldots\theta_k^{a_k-1}\left(\sum_{i=1}^{k}\theta_i * p_{1|i}\right)^{y_1}\left(\sum_{i=1}^{k}\theta_i * p_{2|i}\right)^{y_2}\ldots\left(\sum_{i=1}^{k}\theta_i * p_{k|i}\right)^{y_k} \quad \text{(A.4)}$$

We use the Metropolis-Hastings algorithm to simulate the posterior distribution given in (A.4) (Tierney, 1994). For each random draw from its posterior distribution, we use (A.5) to compute the probability of identifying all the strains actually present on a farm for a given sample size.

$$p = \sum_{i=1}^{k} \theta_i p_{i|i} \quad \text{(A.5)}$$

where $p_{i|i}$ is the probability of observing i strains given there are i strains on a farm.

METROPOLIS-HASTINGS ALGORITHM. There are many types of the Metropolis-Hastings algorithm. The one we used is called the independent Metropolis-Hastings algorithm because the proposal density is independent of the target density. The algorithm can be described as follows:

Suppose at the t^{th} iteration the draw is Θ^t, then the draw of the $(t+1)^{th}$ iteration, Θ^{t+1}, can be generated as follows:

Generate $\Gamma\sim g(\Gamma)$ (proposal density)

Take

$$\Theta^{t+1} = \begin{cases} \Gamma & \text{with probability } \min\left\{\dfrac{\pi(\Gamma \mid Y)\mathrm{g}(\Theta^t)}{\pi(\Theta^t \mid Y)\mathrm{g}(\Gamma)}, 1\right\} \\ \Theta^t & \text{otherwise} \end{cases}$$

The key point of implementing this algorithm is the choice of the proposal density, g(Γ). To achieve a high acceptance probability, we use g(Γ)=Dirichlet(a_1+y_1, a_2+y_2,…, a_k+y_k). To diminish the effect of the starting value, burn-in and thinning were used. To achieve a high precision of estimation, a final sample size of 10,000 was used, though the algorithm converged after about 1000 iterations for our case. The sample means were used to estimate Θ and compute p defined in (A.5). In this way the mean can be used to estimate the probability of observing all strains for a given sample size.

Computation of $p_{j|i}$

Recall that $p_{j|i}$ is the probability of observing j strains given that the flock contains i strains. Suppose there are i strains in a flock, the equal concentrations assumption means a randomly selected isolate has the same probability, 1/i, of being any of the i strains. Suppose there are I strains, at most, in a flock and we take n isolates from this flock. Let X=(x_1,x_2,…,x_i) denote the vector of counts where x_k gives the number of isolates out of n that are from each strain k for k=1,2,.i. Given the equal concentrations assumption, we have the following:

X~Multinomial(n, 1/i, 1/i,… 1/i) (A.6)

In an earlier study (Singer et al., 2000), $p_{j|i}$s were computed by enumerating all the possible outcomes of the multinomial trials. Since they only considered at most 3 strains, there was no problem with implemention of this idea. In this analysis, there are many more strains and isolates to consider. To enumerate all the possible outcomes is practically impossible.

An efficient way to solve this problem is simulation. For any given i, we simulated 1,000,000 X vectors using X~Multinomial (n, 1/i, 1/i,… 1/i). Then we counted the number of X vectors containing 1, 2,…, i nonempty elements. These counts were divided by 1,000,000; the total number of simulations, and used to estimate $p_{j|i}$'s.

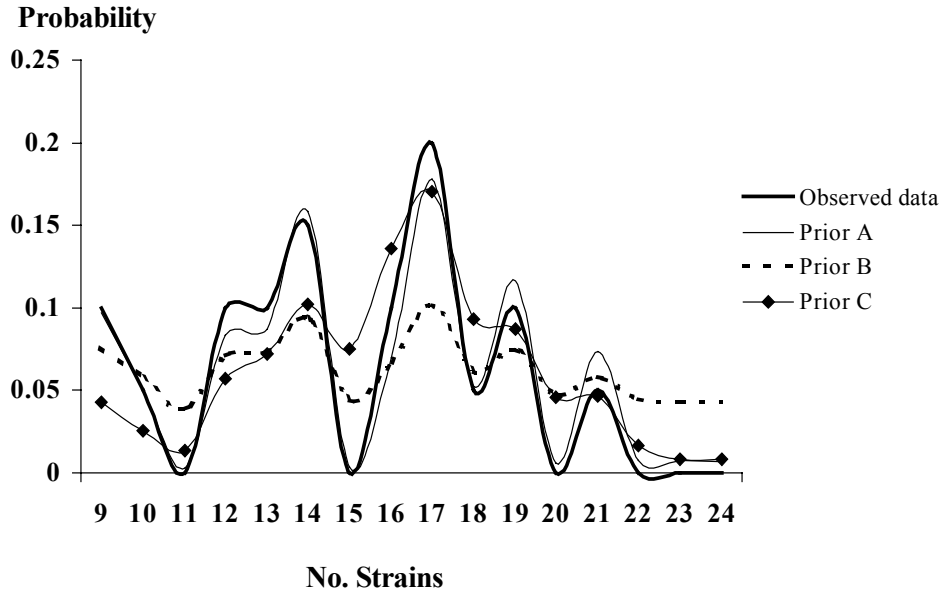
Results

First analysis: Effect of prior probability statements

In the first analysis the uniform prior probability statement with a small prior sample size (i.e., Dirichlet (1/16,1/16,…,1/16) yielded a posterior probability distribution similar to the distribution of observed data (Figure 4.1). The uniform prior probability statement with sample size of 32 assigned probabilities to empty cells in the observed data; however, peaks and nadirs continued to correspond with the number of strains per flock that in observed data.

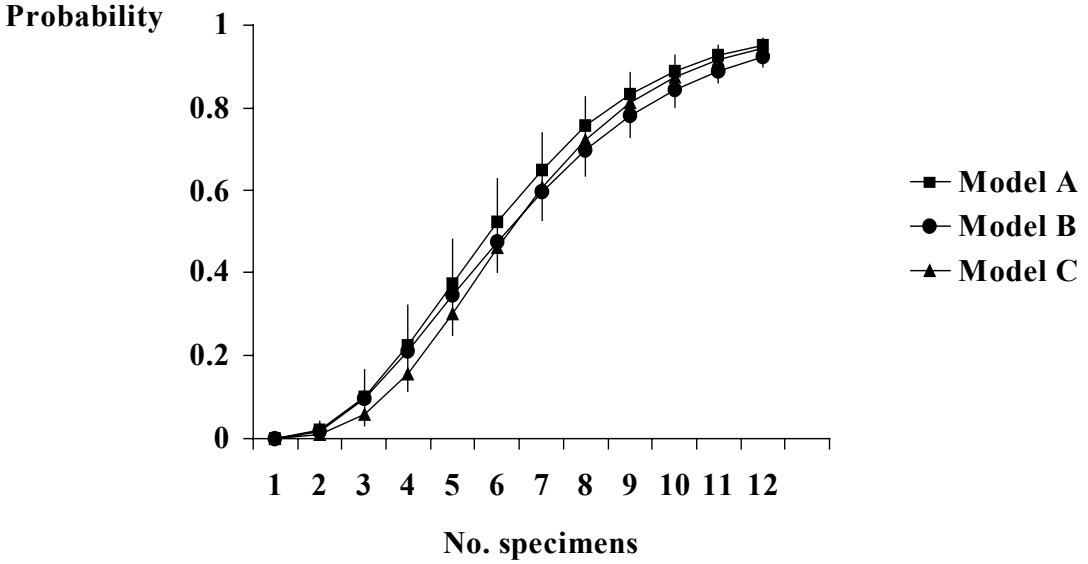
The symmetrically distributed prior probability statement assigned higher probabilities to the midrange of the distribution than to either extreme. Overall, these prior statements were consistent with observed data and had minimal effect on the probability of correctly identifying all strains (Figure 4.2). In each model, mean probabilities of correctly identifying all strains were between 90 and 95% when 12 specimens (96 isolates) were collected per flock.

Figure 4.1. Observed and posterior distributions of the number of strains per flock data for the analyses of 566 isolates with ß-glucuronidase activity for biochemical phenotypes that were observed at least twice in 20 turkey flocks.



NOTES: The dark solid line indicates distribution of actual data from 20 finisher flocks. Prior probability statement A specified equal probability of actual number of strains per flock over the range of 9 to 24 isolates with a prior sample size of one flock, i.e., Dirichlet (1/16, 1/16,…,1/16). Prior probability statement B specified equal probability of the actual number of strains per flock over the range of 9 to 24 isolates with a prior sample size of 32 flocks i.e., Dirichlet (2,2,…,2). Prior probability statement C specified a prior probability with modes at 16 and 17 isolates per flock: Dirichlet (0.32, 0.32, 0.64, 1.28, 1.92, 2.56, 3.52, 5.44, 5.44, 3.52, 2.56, 1.92, 1.28, 0.64, 0.32, 0.32) and a prior sample size of 32 flocks.

Figure 4.2. Effect of three prior probability statements on the probability of correctly identifying selected isolates in a flock by number of fecal specimens collected



NOTES: Analyses pertain to 566 isolates with ß-D-glucuronidase activity and biochemical phenotype that were observed at least twice in 20 flocks. Prior probability statement A specified equal probability of actual number of strains per flock over the range of 9 to 24 isolates with a prior sample size of one flock: Dirichlet (1/16, 1/16,…, 1/16). Prior B specified equal probability of the actual number of strains per flock over the range of 9 to 24 isolates with a prior sample size of 32 flocks: Dirichlet (2, 2,…,2). Prior C specified a distribution with modes at 16 and 17 isolates per flock: Dirichlet (0.32, 0.32, 0.64, 1.28, 1.92, 2.56, 3.52, 5.44, 5.44, 3.52, 2.56, 1.92, 1.28, 0.64, 0.32, 0.32) with a prior sample size of 32 flocks. Smoothed lines connect point estimates for each model. Maximum and minimum 90% Bayesian intervals are indicated by high and low bars.

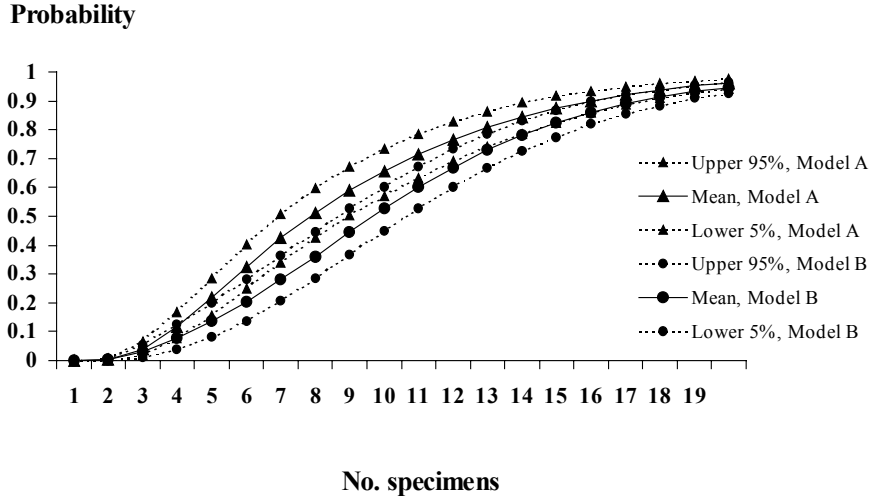
Second analysis: Effect of strain diversity on model

In the analysis restricted to 833 presumptive *E. coli* isolates, a mean probability of 95% was predicted of correctly identifying all strains when 19 specimens were identified per flock. When all 923 strains were included in the model, 21 specimens per flock were required before a mean probability of 95% was reached (Figure 4.3).

Third analysis: Sample size to identify common strains

Five biochemical phenotypes (i.e., PhP-types 2, 5, 10, 14 and 18) accounted for 225 fecal *E. coli* isolates (Table 4.3). The prior probability statements had minimal effect on the estimated probability of correctly identifying widely distributed strains as the number of specimens varied (Figure 4.4). The estimated mean probabilities of correctly identifying the five strains, if present, when one specimen (eight isolates) was collected ranged from 87 % to 89 % and the probabilities when two specimens (16 isolates) were collected exceeded 99%.

Figure 4.3. Probabilities of correctly identifying presumptive *E. coli* isolates if present in a flock by the number of fecal specimens collected for two datasets: 833 isolates with matching biochemical phenotypes and all 923 isolates from 20 finisher flocks



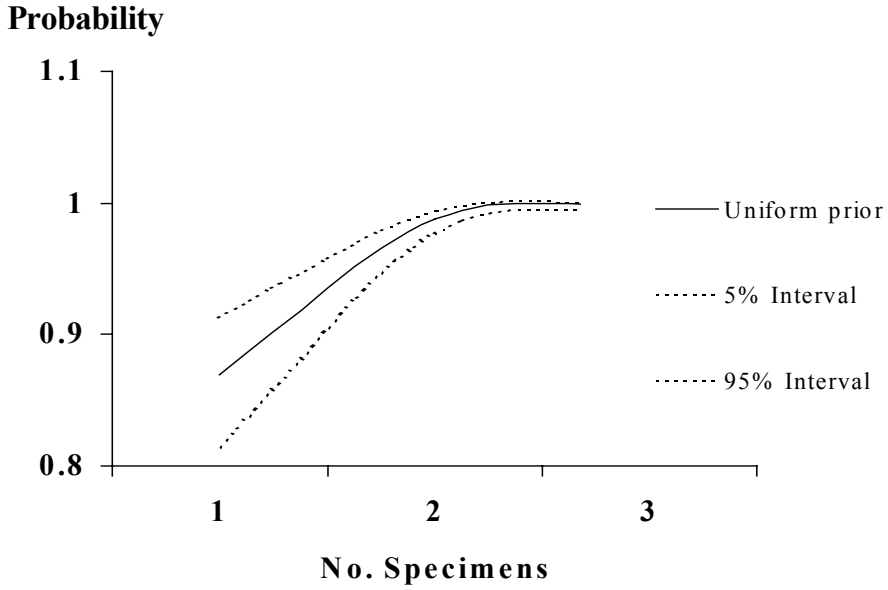
NOTES: Eight colonies were examined per specimen. Analyses were conducted on a subset of all data comprised of 833 isolates with biochemical phenotypes that were observed at least twice and a complete dataset all 923 isolates that were observed in 20 flocks. Both models had prior sample sizes of 2 flocks. The actual range of the number of strains was assumed to be [10, 35] and the prior used is a uniform, Dirichlet (0.867, 0.867) with a "prior sample size" equal to 22.5. Smoothed lines connect point estimates for each model. 90% Bayesian intervals are indicated by dotted lines.

Table 4.3. Distribution of five widely distributed PhP-types of *E. coli* isolates cultured from fecal specimens of finisher turkey flocks

PhP-type	Count	No. flocks	Percent of flocks
PhP-5	95	15	75
PhP-14	50	17	85
PhP-18	32	13	65
PhP-10	19	8	40
PhP-2	29	7	35
All five strains (total)	225	20	100

NOTE: Fecal swabs were collected in an integrated turkey operation from 20 flocks with hens greater than 10 weeks of age. PhP-types are strains with biochemical similarity coefficients $\geq$0.975.

Figure 4.4. Probabilities of correctly identifying all of the five widely distributed fecal *E. coli* strains that are present in a flock by the number of specimens collected



NOTES: The prior probability statement had a uniform distribution Dirichlet (1, 1,…,1) of between one and five strains per flock. Smoothed lines connect point estimates for the model. 90% Bayesian intervals are indicated by dotted lines. The model with prior probability statement Dirichlet (0.25, 1, 2.5, 1, 0.25) also yielded a mean posterior probability of greater than 80% for correctly identifying the five fecal *E. coli* strains that were present in a flock with one specimen (eight colonies) and of 98% with a sample size of two specimens (16 colonies).

Discussion

In the first analysis, prior assumptions and observed data were similar and prior probability statements only modestly influenced estimated sample size to correctly identify *E. coli* strains of interest. The second analysis suggested that there was considerable diversity among fecal *E. coli* isolates from finisher turkey flocks. Based on the models it was predicted that greater than 150 isolates would need to be collected per flock to achieve a 95% mean probability of correctly identifying common fecal *E. coli* strains. From the third analysis it was estimated that five widely distributed biochemical phenotypes of fecal *E. coli* could be identified with 95% probability, if present in a flock, when two specimens (16 colonies) were collected per flock. The markedly different estimates of sample size illustrate the extent to which optimal sample sizes vary depending on the sampling objective. Based on the results, we reject the hypotheses that six

fecal specimens (48 isolates) per flock is an optimal sample size to correctly identify either 1) all fecal *E. coli* strains or 2) the five widely distributed strains.

While the prior probability statements in the first analysis influenced estimated probabilities of the actual number of strains present per flock (Figure 4.1) they were each consistent with the range of the observed data and had minimal effect on the probabilities of correctly identifying all strains in a flock by colony sample size (Figure 4.2). The prior probability statement can be modified in several ways to reflect what is believed to be true and the strength of that belief (Singer et al., 2000). A common criticism is that the prior probability statements introduce subjectivity. An important objective of developing a prior probability statement is to consider what is expected based on previous knowledge and how much conviction is given to that knowledge before a problem statement is made or a hypothesis is tested. This process imposes a structure that diminishes the potential for spurious associations that are encountered when multiple comparisons are made without due consideration given to prior knowledge.

The second analysis provided information regarding the diversity of fecal *E. coli* strains in poultry flocks. The models used in this analysis suggested that a sample size of 48 colonies per flock was unlikely to detect all fecal *E. coli* strains in turkey flocks. A major strength of using this model is that it can be used to update sample size estimates and better allocate laboratory resources for future studies (Singer et al., 2000). By one estimate, 19 specimens (152 colonies) would be needed to achieve a 95 % probability of correctly identifying those fecal *E. coli* strains that were present in the flock at equal concentration. This is more than three times as many specimens as were actually collected for this analysis. It could underestimate the actual number of specimens that would be required to identify all fecal *E. coli* strains in a flock. When insufficient sampling is conducted, the distribution of the observed data is potentially biased toward detecting fewer strains than are present. As a consequence a greater probability is given to correctly identifying all strains with a small sample of bacterial colonies. To update the model, we would recommend collecting additional specimens and making prior assumptions that consider even greater diversity among the strains present per flock. We suspect that such an analysis would predict the need for an even larger sample size to correctly identify the fecal *E. coli* strains in a flock.

In comparison, when the objective of sampling was to detect the most common strains, (the third analysis) the model suggested that two specimens (16 colonies) per flock was a sufficient

sample size. It was noteworthy each of the five strains of interest in this analysis were identified in at least one third of flocks and that three were present in at least half of the flocks that we sampled. The presence of widely dispersed fecal *E. coli* strains has been described (Hinton et al., 1982b; van den Bogaard et al., 2001). It is unclear whether these fecal *E. coli* strains are adapted to a niche within the intestinal tracts of turkeys. It may be worthwhile to evaluate these cosmopolitan fecal *E. coli* strains as potential candidates for competitive exclusion of *Salmonella* (Weinack et al., 1981) and avian pathogenic *E. coli* (La Ragione et al., 2001) from turkey flocks.

Biochemical fingerprinting provides good strain discrimination (Kuhn et al., 1990). Since it is a simple, fast, and automated method it may be particularly suitable for analyzing large numbers of isolates, either alone or in combination with other typing methods. It provided complimentary information to somatic antigen serologic testing; confirming the clustering of some isolates and providing discrimination of other isolates with common surface antigens, phenomena that are well described (Whittam and Wilson, 1988; Caugant et al., 1985). Advantages of biochemical fingerprinting as a method of characterizing *E. coli* include cost, throughput, and the ability to generate data (PhP-types) for all isolates. Fecal *E. coli* could, of course, have been characterized using other laboratory methods. Our objective was primarily to to demonstrate that the Bayesian model is a useful decision-making tool for allocating laboratory resources to efficiently accomplish microbiological sampling objectives.

The Bayesian model that was used requires that an assumption of equal concentrations of strains be made (Singer et al., 2000). While this is a simplistic assumption, it is reasonable. On the one hand, unique strains did comprise seven percent of isolates in the dataset. Furthermore, several strains were distributed across a large proportion of flocks. Nonetheless, the bacterial isolation technique that was used was most likely to detect those fecal *E. coli* strains that were present at highest concentration within a flock (i.e., at concentrations within a range of one or two $\log_{10}$ units from each other).

The Bayesian approach used in these analyses might be particularly beneficial for estimating optimal sample size for common microbial screening procedures. This model has been proposed as a method to estimate the optimal number of drag swabs and colonies to examine per layer flocks when screening for *Salmonella* Enteritidis (Singer et al., 2000; Caldwell et al., 1994). Other potential applications for the model are to estimate probabilities that one, two, or more strains of *Campylobacter jejuni* (Kramer et al., 2000) or *Listeria monocytogenes* are present in a

food specimen. These two applications lie somewhere in the midrange of the spectrum between the *E. coli* cellulitis model (Singer et al. 2000), which was used to examine a homogeneous population, and the fecal *E. coli* model presented here, which was used to examine a heterogeneous population. When ongoing sampling is anticipated the model described here may be used to estimate an optimal sample size and better allocate laboratory resources.

In summary, while prior probability statements influenced the estimated probabilities of actual number of strains per flock, these probabilities had minimal effect on optimal sample size calculations. Fecal *E. coli* isolates from turkey flocks were a heterogeneous population. Based on posterior probability estimates with the Bayesian model, substantially more sampling is required than the 48 isolates that we collected to correctly identify all fecal *E. coli* strains in a flock. In contrast, we estimate that the five most widely distributed strains could be correctly identified, if present, with only two specimens (16 isolates) per flock. Assumptions related to the equal concentration of *E. coli* strains and the interpretation of biochemical phenotypes were necessary for these analyses. The greatest utility of the model may be to update sample size estimates for ongoing microbiological surveys (e.g., screening poultry flocks for *S.* Enteritidis, identifying *Campylobacter jejuni* or *Listeria monocytogenes* in foods).

CHAPTER 5
Commensal and Avian Pathogenic *E. coli*: Current Knowledge, Future Research

Overview

Avian pathogenic *E. coli* infections are a major disease of commercial turkeys (Christiansen et al., 1996; Kriessel et al., 1985; Owings, 1995). These infections contribute to industry losses from drug costs (Lambie et al., 2000), poor production (Pomeroy, 1984; Barnes and Gross, 1997) and regulation to address human infection with antimicrobial resistant foodborne pathogens including *Salmonella* (Glynn et al., 1998), *Campylobacter* (Smith et al., 1999) and the emergence of transmissible plasmids confering bacterial resistance to expanded-spectrum β-lactams (Fey et al., 2000; White et al, 2001). Based on the current state of knowledge (Dho-Moulin and Fairbrother, 1999), two aspects of this infectious disease that should receive particular research priority are the determination of risk factors for flock colonization and infection (Barnes and Gross, 1997) and the identification of markers for avian pathogenic *E. coli* strains (Brown and Curtiss, 1996; Dozois and Curtiss III, 1999; Coulange et al., 2000). These research areas are complementary and may lead to interventions to reduce the burden of colibacillosis infection and the reliance of the turkey industry on antimicrobial therapy to control colibacillosis infections.

Summary of findings

Respiratory syndromes consistent with colibacillosis were the most commonly reported illnesses in the turkey flocks that we studied. Avian pathogenic *E. coli* infections were caused by a limited subgroup of *E. coli* strains that were only rarely detected in the normal fecal flora. Three potential virulence genes; *iss*, K1 and *tsh,* were detected more frequently among *E. coli* isolates from clinical than fecal specimens; however, the pattern of occurrence did not suggest that these genes are suitable markers for discriminating between pathogenic and commensal *E. coli* isolates (Chapter 2). The mean MICs for gentamicin were higher in clinical than fecal isolates and in fecal isolates from brooder units compared to finisher units. These findings raise questions about the rationale for injecting day old poults with gentamicin at the hatchery. Treatment of

colibacillosis was the principal rationale for prescribing antimicrobial drugs. The majority of clinical *E. coli* isolates from this turkey operation were resistant to sulfamethoxazole and tetracycline. Although the rates of resistance for ciprofloxacin, ampicillin, and extended-spectrum β-lactams were less than 50%, there was evidence to suggest that resistance to these antimicrobials could emerge in the future (Chapter 3). Specifically, 43% of clinical isolates were resistant to nalidixic acid. This suggests that *gyr*A point mutations are common among clinical isolates. One additional point mutation, in the *par*C region of the bacterial chromosome, confers complete resistance to the fluoroquinolones ciprofloxacin and enrofloxacin (Hooper and Wolfson, 1993). Furthermore *cmy*2, a plasmid-mediated gene that confers resistance to extended-spectrum β-lactams, was detected among clinical isolates. Penicillin usage provides an advantage to bacteria that carry the *cmy*2 gene (Zhao et al., 2001). This drug was administed to half of the finisher flocks that we sampled.

Our data suggest that a modest surveillance effort (15 isolates per flock and 105 isolates drawn in equal number from seven randomly selected flocks in an operation) can provide accurate and precise estimates of the geometric mean MIC within a flock and across an integrated operation. A model to estimate colony sample size suggested that there is substantial diversity among fecal *E. coli* strains within the sampled turkey flocks. Our analyses also suggested that several widely distributed *E. coli* types occurred in many flocks (Chapter 4). Perhaps some of these widely distributed strains are candidates for use in competive exclusion products.

Unanswered questions

The natural history of infection

The diversity of fecal *E. coli* strains within turkey flocks is a major barrier to microbiological and epidemiological studies of avian pathogenic *E. coli*. A second barrier is that avian pathogenic *E. coli* strains appear to be uncommon components of the normal fecal flora (Barbour et al., 1985; Blanco et al., 1998; Harry and Chubb, 1965; Heller and Drabkin, 1977). These combined factors have hindered studies of the ecology of avian pathogenic *E. coli*. As a consequence the natural history of avian pathogenic *E. coli* infection remains to be resolved. Some researchers view the strains that cause these infections as normal flora that cause

opportunistic infection in susceptible birds while others regard these strains of *E. coli* as specialized pathogens that produce infection in the avian respiratory tract (Blanco, 1997b). Another consequence of the above barriers is that the reservoirs of avian pathogenic *E. coli* strains remain poorly defined (Dho-Moulin and Fairbrother, 1999). For example, the relative contributions of vertical and horizontal routes of transmission still need to be defined (Nivas et al., 1977). Although gentamicin was not used after poults left the hatchery, in this integrated operation, more than half of clinical isolates had elevated MICs for gentamicin. This replicates the findings of other studies (Allan et al. 1993; Bass et al. 1999; Blanco et al., 1997a; Dubel et al. 1982; Irwin et al. 1989). We recommend that the rationale for prophylactic injection of day old birds with this or other antimicrobials (e.g., ceftiofur) be revisited. Since gentamicin was used only in breeding flocks and at the hatchery, the possibility that avian pathogenic *E. coli* may be transmitted, at least in part, via endogenous sources (rather than through the environment) should be considered. Microbiological surveys with differential media containing 1 µg/ml of gentamicin might be used to examine hatch debris such as fluff, sex squeezings, candled out eggs, dead in shells, pips and cull poults (Nivas et al., 1977). The *E. coli* isolates collected in this survey might be characterized using somatic antigen serologic testing (Pomeroy, 1984) and a second laboratory method to improve discrimination. We found biochemical fingerprinting to be a rapid, inexpensive and reliable method for characterizing *E. coli* isolates (PhPlate, 2001).

Risk factors for infection

Although avian pathogenic *E. coli* infections are a leading economic disease of the turkey industry, responsible for approximately one fourth of disease losses (Christiansen et al., 1996; Kriessel et al., 1985; Owings W, 1995), only a few epidemiological studies have been conducted to identify risk factors for avian colibacillosis (Gradel et al., 2001; Pierson et al., 1996b; Tablante et al., 1999). The modernization of the turkey industry in the second half of the twentieth century is considered the global risk factor for the emergence of this infectious disease (Pomeroy et al., 1984). The shift to intensive production and vertical integration produced changes in the turkey production environment including alterations in social stress, air quality, environmental inputs (e.g.; feed, vermin, pathogens) and an emphasis on genetic selection for performance traits at the possible expense of disease resistance traits (Bayyari et al., 1997). These and related changes in

turkey production should be systematically evaluated with carefully designed epidemiologic studies to define risk factors for avian pathogenic *E. coli* infection.

Well-designed epidemiological studies of risk factors will increase the likelihood of identifying cost-effective interventions for avian pathogenic *E. coli* (CDC, 1992). If, for example, the experimental observation that high ammonia concentration predisposes birds to airsacculitis (Anderson et al., 1968) is found under field conditions it provides added rationale for implementing targeted interventions related to air exchange and the control of litter moisture.

Intervention strategies

The vertically integrated turkey industry, which is defined by large-scale production and processing operations is highly amenable to intervention to control avian pathogenic *E. coli* infections (Poss, 1998). Potential interventions for the prevention of avian pathogenic *E. coli* infections include monitoring and biosecurity, environmental controls, flock health programs (e.g., vaccination, competitive exclusion) and selection of disease resistant breeding lines.

Experimental vaccines against avian colibacillosis are described (Abdul-Aziz and el-Sukhon, 1998; Peighambari and Gyles 1998; Roland et al., 1999); however, commercial vaccines for this infection are not widely used. A limitation of most experimental vaccines is that they are directed against a single pathogenic clone (Roland et al., 1999). Because a variety of avian pathogenic *E. coli* clones are known to exist (White et al., 1993b), a commercial vaccine should be multivalent. It will, therefore be important to identify protein(s) expressed by and specific to a large proportion of avian pathogenic *E. coli* clones. Candidate proteins should elicit a protective immune response. Ideally, such protein(s) would be expressed early in infection. Efforts to identify unique sequence islands of avian pathogenic *E. coli* may facilitate this effort (Brown and Curtiss III, 1996; Dozois and Curtiss III, 1999; Coulange et al., 2000).

Competitive exclusion has been proposed as a strategy to prevent salmonellosis (Weinack et al., 1981) as well as avian pathogenic *E. coli* infections (LaRagione et al., 2001). In the latter study, inoculation of specific pathogen free chicks with *Bacillus subtilis* spores competitively excluded *E. coli* O78 from the intestines and suppressed evidence of colibacillosis infection (La Ragione et al., 2001). The number of colony forming units of the bacillus decreased over time and a protective effect was not evident after five days. In our study, several fecal *E. coli* strains were distributed across multiple turkey flocks. Perhaps one or more of the five ubiquitous fecal

E. coli strains that we identified (Chapter 4) have potential to competitively exclude avian pathogenic *E. coli* from poultry intestines (Heller and Drabkin, 1977). Ubiquitous *E. coli* strains are of interest because they may be maintained in the intestinal tracts of turkeys.

Studies of antimicrobial use and resistance

Fecal *E. coli* are an excellent indicator organism for studying the relationship between antimicrobial usage and resistance in poultry production. Fecal *E. coli* strains are abundant in poultry environments (van den Bogard et al., 2001), easy to isolate (Pezzlo, 1992), and do not require extensive confirmatory testing (Dunlop et al., 1999). Field studies of fecal *E. coli* could provide information on the development and duration of antimicrobial resistance (Hinton et al., 1982a). Fecal *E. coli* is also an attractive indicator organism for the study of antimicrobial resistance because it is considered to be a commensal organism rather than a pathogen of humans or poultry. As a consequence, data on the dynamics of antimicrobial resistance in commensal *E. coli* carry less implications regarding liability than would data regarding resistance of *Salmonella*, *Campylobacter*, or avian pathogenic *E. coli*. Based on our experience several modifications are proposed to increase the power of studies relating antimicrobial usage to resistance without an equal increase in laboratory workload.

Our analyses (Chapter 3) suggest that stable estimates of geometric mean MICs can be obtained with fewer than the 48 fecal isolates that were collected per flock. Indeed, when 15 or more isolates were drawn per flock, estimated geometric mean MICs were similar to the overall mean. This suggests that reasonable estimates of mean MIC values could have been obtained for two or three times as many flocks with the same laboratory resources that were used to sample 20 flocks. This type of information is critical for the efficient allocation of scarce laboratory resources (Berkelman et al., 1994). The resulting surveillance data would be of value to poultry veterinarians. The data could be used to rotate therapeutic antimicrobial drugs in an integrated operation to prevent MICs from crossing over a predetermined MIC threshold. The use of surveillance data to preemptively rotate antimicrobial drugs could extend the viability of approved antimicrobial drugs. This is an important consideration since very few new drug approvals are anticipated for poultry production in the foreseeable future.

The effect of antimicrobial usage on resistance in fecal *E. coli* may be most evident in the days after usage ceases. Resistance to therapeutic antimicrobial drugs has been shown to increase

with time and to peak several days after withdrawal (Hinton et al., 1982a). In our small field sample, most antimicrobial therapy occurred during the brooder period of the turkey production cycle. This may have related to the age at which turkeys were affected by disease, cost-related disincentives to use of antimicrobial agents with increasing flock age or efforts to avoid antimicrobial residues at processing. If the relationship between flock age and antimicrobial usage is typical, it has implication for the design of field studies since the greatest effect of antimicrobial usage on geometric mean MIC would be expected in brooder flocks, and particularly within several days after withdrawal of the antimicrobial drug. A more modest effect between usage and resistance might be anticipated for isolates from market age flocks.

A microplate based MIC broth dilution panel, such as the one that was used to characterize our *E. coli* isolates, has several advantages over a breakpoint panel. First, it can provide data over a broad MIC range. This permits shifts in MICs to be detected before a breakpoint threshold is reached. We recommend that MIC panels be designed with greater MIC ranges to avoid truncation at either the low or high end of the MIC range. This can be accomplished by restricting the MIC panel to antimicrobials that are used in the study population (e.g., a poultry production operation). In poultry the antimicrobial classes of interest are aminoglycosides, fluoroquinolones, sulfa drugs and tetracyclines. There is concern that *cmy*2 gene-mediated resistance is emerging to extended-spectrum β-lactams. It may, therefore be prudent to include a drug in this class on an MIC panel (Fey et al., 2000). In the 1990s, the addition of chloramphenicol to the gram negative enteric bacterial panel was recommended to facilitate identification of *Salmonella* Typhimurium DT 104 (Glynn et al., 1998). The 96 well microplate configuration limits the number of antimicrobial drugs and the MIC ranges that can be measured on a microplate-based MIC panel. For this reason, an MIC panel must be carefully designed and updated to address current surveillance objectives pertaining to antimicrobial resistance.

Strain markers for pathogens

Clinical isolates from turkeys with colibacillosis are a valuable epidemiological resource. If possible, these isolates should be collected, characterized, and maintained as a library for future study (Blanco et al., 1998; Dho-Moulin and Fairbrother, 1999). Somatic antigen serologic testing is the most widely recognized method of characterizing avian pathogenic *E. coli* (Orskov et al., 1977). Since there is heterogeneity within (Caugant et al., 1985) and homogeneity across

serogroups (Whittam and Wilson, 1988), it may be useful to conduct somatic antigen serologic testing in conjunction with another method of characterization. We found that biochemical fingerprinting provided information that was complimentary to somatic antigen test results (PhPlate, 2001). Microbiologists in other laboratories have reported favorable results with other characterization techniques including ERIC-PCR (Carvalho de Moura et al., 2001; Dombek et al., 2000), RAPD-PCR (Chansiripornchai et al., 2001; Maurer et al., 1998b) and pulsed-field electrophoresis (van den Bogaard et al., 2001). Characterization of clinical isolates is an important source of information on the pathogenic strains that are present in a poultry operation. Conducted on an ongoing basis, it can provide insight into the spatial and temporal flow of avian pathogenic *E. coli* strains. If isolates are routinely collected and rapidly characterized it may even facilitate investigation of potential sources of avian pathogenic *E. coli*. Such information, on the ecology of avain pathogenic *E. coli* strains, would be a valuable contribution to understanding of avian pathogenic *E. coli*. Furthermore, clinical isolates can also provide information on previous antimicrobial resistance patterns encountered in an integrated operation. This information can be used to make informed decisions regarding antimicrobial therapy for flocks affected with colibacillosis (FDA, 2001).

Current knowledge of the mechanism by which avian pathogenic *E. coli* cause disease remains piecemeal (Dozois and Curtiss III, 1999). It is known that a limited set of *E. coli* strains cause most colibacillosis infections and potential virulence genes have been identified; however, the context in which these genes interact to produce the syndrome of avian colibacillosis remains vaguely defined (Dho-Moulin and Fairbrother, 1999). Plasmid mediated virulence determinants have received much attention (Doetkott et al, 1996). Twelve unique chromosomal sequences that cumulatively comprise 300-400 kb were detected in the avian pathogenic *E. coli* O78 strain X ^{7122} by subtractive hybridization with *E. coli* K12 (Brown and Curtiss III, 1996). Other researchers have identified additional unique chromosomal fragments (Coulange et al., 2000). These include sequences with homology to genes encoding FruA (a protein involved in fructose metabolism) and TktA (a transketolase) of *E. coli*, Gp2 a terminase of phage 21, and, RatA a suspected suface protein with repeat structure first described in a pathogenicity island of *Salmonella* affecting warm-blooded animals. Coulange et al. (2000) described an additional chromosomal fragment with no homology to sequences in current databases.

These and similar sequences are currently being examined by several laboratories (Brown and Curtiss III, 1996; Dozois and Curtiss III, 1999; Coulange, 2000). Genes that are suspected to occur on these unique sequence islands are listed in Table 5.1. The pathogenicity islands of uropathogenic and enteropathogenic *E. coli* have been meticulously characterized (Dozois and

Table 5.1. Potential components of unique sequence islands of avian pathogenic *E. coli* strains and the relevant citations

Possible constituents, unique sequences of APEC[a] strains	Citation
K1 group II capsule (sugar island)	Vimr, 1991
IntI1(Class 1 integron)	Bass et al., 1999
tsh gene encoding temperature-sensitive hemagglutinin	Brown and Curtiss III, 1996 Dozois et al., 2000
rfb cluster involved in O78 antigen synthesis	Brown and Curtiss III, 1996
Genes encoding protein homologous to TktA, FruA (*E. coli*), Gp2 (phage 21), RatA of (S. enterica) and sequence with no similarity to those in the current database	Coulange et al., 2000
Genes associated with adhesion fimbriae	La Ragione et al., 2000 Arne et al., 2000
Pathogenicity Island I, II and LEE insertion sites	Brown and Curtiss III, 1996
tRNA primers for selC site downstream from LEE and PAI I	Oswald et al., 2000
Phage integration site of *bor*, outer membrane lipoprotein	Barondess et al., 1995
Iron uptake genes	Vokes et al., 1999

[a]APEC = Avian pathogenic *E. coli*

Curtiss III, 1999). The slow pace of progress in characterization of avian pathogens may relate to funding, complexity, and competing research priorities.

The unique sequence islands of avian pathogenic *E. coli* may provide insight into the genes that contribute to respiratory and invasive bacterial infections in general and avian pathogenic *E. coli* in particular. The progress of research may be facilitated by parallel research conducted with enteropathogenic *E. coli* (Kaper et al., 1997). The resulting insights into the molecular bases of pathogenesis could lead to development of new controls for avian pathogenic *E. coli* infections. Anticipated scientific communications may provide leads on oligonucleotide probes that can be used to identify unique sequence islands that are shared by the various avian pathogenic *E. coli* clones (Brown and Curtiss III, 1996; Coulange, 2000, Dozois and Curtiss III, 1999). Our library of clinical isolates from turkeys is well suited for studies to identify conserved unique sequences of avian pathogenic *E. coli* strains because their somatic antigens, biochemical and antimicrobial

susceptibility characteristics and potential virulence determinants are known. The principal objective of the recommended research would be to identify unique genetic sequences (markers) that are common to avian pathogenic *E. coli*.

The identification of genomic sequences that are common in pathogenic clones and rare in commensal *E. coli* would provide a tool for microbiological surveys and epidemiological studies of avian pathogenic *E. coli* (Pfaff-McDonough et al., 2000). If a reliable marker for pathogenicity were identified, it could be used to estimate the proportion of flocks that are colonized with *E. coli* strains containing the marker or examine risk factors for the progression from colonization to clinical avian pathogenic *E. coli* infection. Such a marker might also reduce cost and workload requirements to study sources of avian pathogenic *E. coli* strains by eliminating background commensal *E. coli* from the pool of isolates that undergo further laboratory characterization. As a cautionary note, the pathogenesis of bacterial infections is often multifactorial and the presence or absence of a single determinant may not be a definitive marker for virulence (Mobley et al., 1994).

Recommendations for future research

Research to identify markers that discriminate between pathogenic and commensal avian *E. coli* strains should remain a high priority among poultry disease issues. Anticipated publications regarding the characteristics of unique sequence islands within the genome of selected avian pathogenic *E. coli* strains may provide clues to identify these elusive markers. Epidemiological studies are also recommended to evaluate potential risk factors for colonization and for outbreaks of disease caused by these strains. The results of these types of studies may help the industry officials to implement cost-effective strategies to prevent avian pathogenic *E. coli* infections.

In summary, there are intriguing hypotheses to be addressed regarding avian colibacillosis at both the molecular- and population-levels. These areas of research are complimentary to one another, addressing unanswered questions regarding the interrelationship between the avian host, the poultry environment, and the pathogen responsible for avian pathogenic *E. coli* infection.

References

Abdul-Aziz TA, el-Sukhon SN. Chickens hyperimmunized with *Escherichia coli* J5 strain are protected against experimental challenge with *Escherichia coli* O78 serotype. Vet Res Commun 1998; 22:7-9.

al-Homidan A, Robertson JF, Petchey Effect of environmental factors on ammonia and dust production broilers performance. Br Poult Sci 1998; 39:S9-10.

Allan BJ, van den Hurk JV, Potter AA. Characterization of *Escherichia coli* isolated from cases of avian colibacillosis. Can J Vet Res 1993; 57:146-51.

Anderson DP, Wolfe RR, Cherms FL, Roper WE. Influence of dust and ammonia on the development of air sac lesions in turkeys. Am J Vet Res 1968; 29:1049-58.

Arne P, Marc D, Bree A, Schouler C, Dho-Moulin M. Increased tracheal colonization in chickens without impairing pathogenic properties of avian pathogenic *Escherichia coli* MT78 with a fimH deletion. Avian Dis 2000; 44:343-55.

Bager F. DANMAP: monitoring antimicrobial resistance in Denmark. International J Antimicrobial Agents 2000; 14:271-74.

Bailey JS, Lyon BG, Lyon CE, Windham WR. The microbiological profile of chilled and frozen chicken. J Food Prot 2000; 63:1228-30.

Barbour EK, Nabbut NH, Al-Nakhli HM. Use of epidemiologic markers to identify the source of *Escherichia coli* infections in poultry. Am J Vet Res 1985; 46:989-91.

Barnes HJ, Gross WB. Colibacillosis, In: Diseases of Poultry, 10th ed. Calnek BW, Barnes HJ, Beard CW, McDougald LR, Saif YM; eds. Iowa State University Press. Ames, IA. 1997; pp. 131-41.

Barondess JJ, Beckwith J. *Bor* gene of phage lambda, involved in serum resistance, encodes a widely conserved outer membrane lipoprotein. J Bacteriol 1995; 177:1247-53.

Bass L, Liebert CA, Lee MD, Summers AO, White DG, Thayer SG, Maurer JJ. Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in Avian *Escherichia coli*. Animicrobial Agents and Chemotherapy 1999; 43: 2925-9.

Bayes T. An Essay Toward Solving a Problem in the Doctrine of Chances. Philos. Trans. R. Soc. London 1763: 70-418. (Reprinted in Biometrika 1958; 45: 293-315.)

Bayyari GR, Huff WE, Rath NC, Balog JM, Newberry LA, Villines JD, Skeeles JK, Anthony NB, Nestor KE. Effect of the genetic selection of turkeys for increased body weight and egg production on immune and physiological responses. Poult Sci 1997; 76: 289-96.

Berkelman RL, Bryan RT, Osterholm MT, LeDuc JW, Hughes JM. Infectious disease surveillance: a crumbling foundation. Science 1994; 264:368-70.

Binns MM, Davies DL, Hardy KG. Cloned fragments of the plasmid ColV, I-K94 specifying virulence and serum resistance. Nature 1979; 279:778-81.

Blanco JE, Blanco M, Mora A, Blanco J. Prevalence of bacterial resistance to quinolones and other antimicrobials among avian *Escherichia coli* strains isolated from septicemic and healthy chickens in Spain. J Clin Microbiol 1997; 35:2184-5.

Blanco JE, Blanco M, Mora A, Blanco J. Production of toxins (enterotoxins, verotoxins, and necrotoxins) and colicins by *Escherichia coli* strains isolated from septicemic and healthy chickens: relationship with in vivo pathogenicity. J Clin Microbiol 1997; 35:2953-7.

Blanco JE, Blanco M, Mora A, Jansen WH, Garcia V, Vazquez ML, Blanco J. Serotypes of *Escherichia coli* isolated from septicaemic chickens in Galicia (northwest Spain) Vet Microbiol 1998; 31:229-35.

Bloch CA, Rode CK. Pathogenicity island evaluation in *Escherichia coli* K1 by crossing with laboratory strain K-12. Infect Immun 1996; 64:3218-23.

Bradford PA, Petersen PJ, Fingerman IM, White DG. Characterization of expanded-spectrum cephalosporin resistance in *E. coli* isolates associated with bovine calf diarrhoeal disease. J Antimicrob Chemother 1999; 44:607-10.

Bree A, Dho M, Lafont JP. Comparative infectivity for axenic and specific-pathogen-free chickens of O2 *Escherichia coli* strains with or without virulence factors. Avian Dis 1989; 33:134-9.

Bretthorst GL. An introduction to model selection using probability theory as logic. In: Maximum Entropy and Bayesian Methods 1993. G. Heidbreder ed., Kluwer Academic Publishers. Dordrecht, the Netherlands. 1994. pp 7-22.

Brown PK, Curtiss III R. Unique chromosomal regions associated with virulence of an avian pathogenic *Escherichia coli* strain. Proc Natl Acad Sci USA 1996; 93:11149-54.

Caldwell DJ, Hargis BM, Corrier DE, Williams JD, Vidal L, DeLoach JR. Predictive value of multiple drag-swab sampling for the detection of *Salmonella* from occupied or vacant poultry houses. Avian Dis 1994; 38:461-6.

Carlson HC, Whenham GR. Coliform bacteria in chicken broiler house dust and their possible relationship to *coli*-septicemia. Avian Dis 1968; 12:297-302.

Carvalho de Moura AC, Irino K, Vidotto MC. Genetic variability of avian *Escherichia coli* strains evaluated by enterobacterial repetitive intergenic consensus and repetitive extragenic palindromic polymerase chain reaction. Avian Dis 2001; 45:173-181.

Caugant DA, Levin BR, Orskov I, Orskov F, Svanborg EC, Selander RK. Genetic diversity in relation to serotype in *Escherichia coli*. Infect Immun 1985; 49:407-13.

CDC. A framework for assessing the effectiveness of disease and injury prevention. MMWR Supplement 1992; 41: Number RR-3.

Chaffer M, Heller ED, Schwartsburd B. Relationship between resistance to complement, virulence and outer membrane protein patterns in pathogenic *Escherichia coli* O2 isolates.Vet Microbiol 1999; 64:323-32.

Chansiripornchai N, Ramasoota P, Sasipreeyajan J, Svenson SB. Differentiation of avian pathogenic *Escherichia coli* (APEC) strains by random amplified polymorphic DNA (RAPD) analysis. : Vet Microbiol 2001;80:75-83.

Christiansen KH, Hird DW, Snipes KP, Danaye-Elmi C, Palmer CW, McBride MD, Utterback WW. California National Animal Health Monitoring System for meat turkey flocks - 1988-89 pilot study: Management practices, flock health, and production. Avian Dis 1996; 40:278-84.

Chuba PJ, Leon MA, Banerjee A, Palchaudhuri S. Cloning and DNA sequence of plasmid determinant *iss* coding for increased serum survival and surface exclusion, which has homology with lambda DNA. Mol Gen Genet. 1989; 216:287-92.

Cloud SS, Rosenberger JK, Fries PA, Wilson RA, Odor EM. In vitro and in vivo characterization of avian *Escherichia coli*. I. Serotypes, metabolic activity, and antibiotic sensitivity. Avian Dis 1985; 29:1084-93.

Coulange F, Leroy-Sétrin S, Schouler C, Dho-Moulin M. Isolation of genomic fragments specific for avian pathogenic *Escherichia coli* (APEC). Proceedings of the 100th General Meeting of the American Society for Microbiology. Los Angeles, CA 2000, Abstract B64.

David BP, Purushothaman V, Venkatesan RA. Comparison of plasmid profile analysis, antibiogram testing, resistotyping and biotyping in the identification of *Escherichia coli* isolates from poultry. Vet Rec 1991; 129:94-7.

De las Casas E, Pomeroy BS, Harein PK. Infection and quantitative recovery of *Salmonella* typhimurium and *Escherichia coli* from within the lesser mealworm, *Alphitobius diaperinus* (panzer). Poult Sci 1968; 47:1871-5.

Dho-Moulin M, van den Bosch JF, Girardeau JP, Bree A, Barat T, Lafont JP. Surface antigens from *Escherichia coli* O2 and O78 strains of avian origin. Infect Immun 1990; 58:740-5.

Dho-Moulin M, Fairbrother JM. Avian pathogenic *Escherichia coli* (APEC). Vet Res 1999; 30:299-316.

Doetkott DM, Nolan LK, Giddings CW, Berryhill DL. Large plasmids of avian *Escherichia coli* isolates. Avian Dis 1996; 40:927-30.

Dombek PE, Johnson LK, Zimmerley ST, Sadowsky MJ. Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. Appl Environ Microbiol 2000; 66:2572-7.

Dozois CM, Dho-Moulin M, Bree A, Fairbrother JM, Desautels C, Curtiss III R. Relationship between the *tsh* autotransporter and pathogenicity of avian *Escherichia coli* and localization and analysis of the *tsh* genetic region. Infect Immun 2000; 68:4145-54.

Dozois CM, Fairbrother JM, Harel J, Bosse M. Pap-and pil-related DNA sequences and other virulence determinants associated with *Escherichia coli* isolated from septicemic chickens and turkeys. Infect Immun 1992; 60:2648-56.

Dubel JR, Zink DL, Kelley LM, Naqi SA, Renshaw HW. Bacterial antibiotic resistance: frequency of gentamicin-resistant strains of *Escherichia coli* in the fecal microflora of commercial turkeys. Am J Vet Res 1982; 43:1786-9.

Dunlop RH, McEwens SA, Meek AH, Friendship RM, Black WD, Clarke RC. Sampling considerations for herd-level measurement of faecal *Escherichia coli* antimicrobial resistance in finisher pigs. Epidemiol Infect 1999; 122:485-9.

Ellis MG, Arp LH, Lamont SJ. Serum resistance and virulence of *Escherichia coli* isolated from turkeys. Am J Vet Res 1988; 49:2034-7.

Emery DA, Nagaraja KV, Shaw DP, Newman JA, White DG. Virulence factors of *Escherichia coli* associated with *coli*septicemia in chickens and turkeys. Avian Dis 1992; 36:504-11.

Fantinatti F, Silveira WD, Castro AF. Characteristics associated with pathogenicity of avian septicaemic *Escherichia coli* strains. Vet Microbiol 1994:41:75-86.

Fey PD, Safranek TJ, Rupp ME, Dunne EF, Ribot E, Iwen PC, Bradford PA, Angulo FJ, Hinrichs SH. Ceftriaxone-resistant *Salmonella* infection acquired by a child from cattle. N Engl J Med 2000; 342:1242-9.

Ficken MD, Edwards JF, Lay JC. Clearance of bacteria in turkeys with *Bordetella avium*-induced tracheitis. Avian Dis 1986;30:352-7.

Ficken MD, Edwards JF, Lay JC, Tveter DE. Tracheal mucus transport rate and bacterial clearance in turkeys exposed by aerosol to La Sota strain of Newcastle disease virus. Avian Dis 1987;31:241-8.

FDA, Center for Veterinary Medicine. Judicious Use of Antimicrobials for Poultry Veterinarians. Worldwide Web: http://www.fda.gov/cvm/fsi/JUPOULTRY.pdf. Accessed October 6, 2001.

FDA Task Force on Antimicrobial Resistance. 2000. Rockville, MD. Worldwide Web: http://www.fda.gov/oc/antimicrobial/taskforce2000.html. Accessed October 6, 2001.

Foley SL, Horne SM, Giddings CW, Robinson M, Nolan LK. *Iss* from a virulent avian *Escherichia coli*. Avian Dis 2000; 44:185-191.

Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo FJ. Emergence of multidrug-resistant *Salmonella enterica* serotype typhimurium DT104 infections in the United States. N Engl J Med 1998; 338:1333-8.

Gomis SM, Riddell C, Potter AA, Allan BJ. Phenotypic and genotypic characterization of virulence factors of *Escherichia coli* isolated from broiler chickens with simultaneous occurrence of cellulitis and other colibacillosis lesions. Can J Vet Res 2001; 65:1-6.

Gradel KO, Feld NC, Andersen JS. Serologic reactions against *Salmonella* in samples from broiler parent stock with and without preceding colibacillosis: a case-control study. Avian Dis 2001:45:486-91.

Gross WB. The development of "air sac disease." Avian Dis 1961; 5:431-9.

Gross WB, Siegel PB. Long-term exposure of chickens to three levels of social stress. Avian Dis 1981; 25:312-25.

Guyer DM, Nereus IV WG, Harry L, Mobley T. Secreted proteins and other features specific to uropathogenic *Escherichia coli*. J Infect Disease 2001; 183:S32-5.

Harry EG. A study of 119 outbreaks of coli-septicaemia in broiler flocks. Vet Rec 1964; 76:443-9.

Harry EG, Chubb HL. Relationships between certain biochemical characteristics and pathological activity in avian strains of *E. coli*. J Comp Path Therapeutics 1964; 74:180-7.

Hayes JR, Carr LE, Mallinson ET, Douglass LW, Joseph SW. Characterization of the contribution of water activity and moisture content to the population distribution of *Salmonella* spp. in commercial poultry houses. Poult Sci 2000; 79:1557-61.

Heller ED, Drabkin N. Some characteristics of pathogenic *Escherichia coli* strains. Br Vet J 1977; 133:572-8.

Heller ED, Smith HW. The incidence of antibiotic resistance and other characteristics amongst *Escherichia coli* strains causing fatal infection in chickens: the utilization of these characteristics to study the epidemiology of the infection. J Hyg (Lond) 1973; 71:771-81.

Hemsley RV, Barnum DA, Ingram DG. Biochemical and serological studies of avian strains of *Escherichia coli*. Avian Dis 1967; 11:90-7.

Hemsley LA, Harry EG. Coliform pericarditis (colisepticaemia) in broiler chickens: A three year study on one farm. Vet Rec 1965; 77:103-7.

Hinton M, Al-Chalaby ZA, Allen V, Linton AH. The persistence of drug resistant *Escherichia coli* in the intestinal flora of healthy broiler chicks. J Hyg (Lond) 1982; 89:269-78.

Hinton M, Allen V, Linton AH. The biotyping of *Escherichia coli* isolated from healthy farm animals. J Hyg (Lond) 1982; 88:543-55.

Hooper DC, Wolfson JS. Mechanisms of bacterial resistance to quinolones. In: Quinolone antimicrobial agents, 2nd ed. Hooper DC, Wolfson JS eds. American Society for Microbiology. Washington, DC. 1993; pp. 97-118.

Huff GR, Huff WE, Balog JM, Rath NC. Effect of early handling of turkey poults on later responses to multiple dexamethasone *Escherichia coli* challenge. 2. Resistance to air sacculitis and turkey osteomyelitis complex. Poult Sci 2001; 80:1314-22.

Ike K, Kawahara K, Danbara H, Kume K Serum resistance and aerobactin iron uptake in avian *Escherichia coli* mediated by conjugative 100-megadalton plasmid. J Vet Med Sci 1992; 54:1091-8.

Irwin RJ, McEwen SA, Clarke RC, Meek AH. The prevalence of verocytotoxin-producing *Escherichia coli* and antimicrobial resistance patterns of nonverocytotoxin-producing *Escherichia coli* and *Salmonella* in Ontario broiler chickens. Can J Vet Res 1989; 53:411-8.

Jann K, Jann B. Capsules of *Escherichia coli*, expression and biological significance. Can J Microbiol 1992; 38:705-10.

Kaper JB, McDaniel TK, Jarvis KG, Gomez-Duarte O. Genetics of virulence of enteropathogenic *E. coli*. Adv Exp Med Biol 1997; 412:279-87.

Katouli M, Lund A, Wallgren P, Kuhn I, Soderlind O, Mollby R. Phenotypic characterization of intestinal *Escherichia coli* of pigs during suckling, postweaning, and fattening periods. Appl Environ Microbiol 1995; 61:778-83.

Keyes K, Hudson C, Maurer JJ, Thayer S, White DG, Lee MD. Detection of florfenicol resistance genes in *Escherichia coli* isolated from sick chickens. Antimicrob Agents Chemother 2000; 44:421-4.

Kramer JM, Frost JA, Bolton FJ, Wareing DR. *Campylobacter* contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection. J Food Prot 2000; 63:1654-9.

Kriessel KA, Scott C, Rock C. Minnesota turkey deaths and losses. Minnesota Department of Agriculture. St. Paul, MN. 1985; pp. 1-4.

Kuhn I, Brauner A, Mollby R. Evaluation of numerical typing systems for *Escherichia coli* using the API 50 CH and the PhP-EC systems as models. Epidemiol Infect 1990; 105:521-31.

Kumar MC, Olson HR, Ausherman LT, Thurber WB, Field M, Hohlstein WH, Pomeroy BS. Evaluation of monitoring programs for *Salmonella* infection in turkey breeding flocks. Avian Dis 1972; 16:644-8.

La Ragione RM, Casula G, Cutting SM, Woodward MJ. *Bacillus subtilis* spores competitively exclude *Escherichia coli* O78:K80 in poultry. Vet Microbiol 2001; 79:133-42.

La Ragione RM, Cooley WA, Woodward MJ. The role of fimbriae and flagella in the adherence of avian strains of *Escherichia coli* O78:K80 to tissue culture cells and tracheal and gut explants. J Med Microbiol 2000; 49:327-38.

Lafont JP, Dho M, d'Hauteville HM, Brée A, Sansonetti PJ. Presence and expression of aerobactin genes in virulent avian strains of *Escherichia coli*. Infect Immun 2000; 55:193-7.

Lambie N, Ngeleka M, Brown G, Ryan J. Retrospective study on *Escherichia coli* infection in broilers subjected to postmortem examination and antibiotic resistance of isolates in Trinidad. Avian Dis 2000; 44:155-60.

Larsen CT, Domermuth CH, Sponenberg DP, Gross WB. Colibacillosis of turkeys exacerbated by hemorrhagic enteritis virus. Laboratory studies. Avian Dis 1985; 29:729-32.

Larsen CT, Pierson FW, Gross WB. Effect of dietary selenium on the response of stressed and unstressed chickens to *Escherichia coli* challenge and antigen. Biol Trace Elem Res 1997; 58:169-76.

Leitner G, Heller ED. Colonization of *Escherichia coli* in young turkeys and chickens. Avian Dis 1992; 36:211-20.

Loken KI, Culbert KH, Solee RE, Pomeroy BS. Microbiological quality of protein feed supplements produced by rendering plants. Appl Microbiol 1968; 16:1002-5.

Linggood MA, Roberts M, Ford S, Parry SH, Williams PH. Incidence of the aerobactin iron uptake system among *Escherichia coli* isolates from infections of farm animals. J Gen Microbiol 1987; 33:835-42.

Mallinson ET, Tate CR, Miller RG, Bennett B, Russek-Cohen E. Monitoring poultry farms for *Salmonella* by drag-swab sampling and antigen-capture immunoassay. Avian Dis 1989; 33:684-90.

Maurer JJ, Brown TP, Steffens WL, Thayer SG. The occurrence of ambient temperature-regulated adhesins, curli, and the temperature-sensitive hemagglutinin *tsh* among avian *Escherichia coli*. Avian Dis 1998; 42:106-18.

Maurer JJ, Lee MD, Lobsinger C, Brown T, Maier M, Thayer SG. Molecular typing of avian *Escherichia coli* isolates by random amplification of polymorphic DNA. Avian Dis 1998; 42:431-51.

Minshew BH, Jorgensen J, Swanstrum M, Grootes-Reuvecamp GA, Falkow S. Some characteristics of *Escherichia coli* strains isolated from extraintestinal infections of humans. J Infect Dis 1978; 137:648-54.

Mobley HL, Island MD, Massad G. Virulence determinants of uropathogenic *Escherichia coli* and *Proteus mirabilis*. Kidney Int Suppl 1994;47:S129-36.

Moreno MA, Domínguez L, Teshager T, Herrero IA, Porrero MC and The VAV Network, Antibiotic resistance monitoring: the Spanish programme. International J Antimicrobial Agents 2000; 14:285-90.

Morley PS, Bolte D, Wittum TE. Effect of varying sampling strategies within-animals on population estimates for antimicrobial drug resistance in non-type-specific *E. coli*. Proceedings of the International Society for Veterinary Epidemiology. 2000. Breckenridge, CO. Abstract 607.

Nagaraja KV, Emery DA, Jordan KA, Sivanandan V, Newman JA, Pomeroy BS. Effect of ammonia on the quantitative clearance of *Escherichia coli* from lungs, air sacs, and livers of turkeys aerosol vaccinated against *Escherichia coli*. Am J Vet Res 1984; 45:392-5.

Naveh MW, Zusman T, Skutelsky E, Ron EZ. Adherence pili in avian strains of *Escherichia coli*: effect on pathogenicity. Avian Dis 1984; 28:651-61.

National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard. NCCLS Document M31-A (ISBN 1-56238-377-9). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 USA 1999.

Newberry LA, Skeeles JK, Kreider DL, Beasley JN, Story JD, McNew RW, Berridge BR. Use of virulent hemorrhagic enteritis virus for the induction of colibacillosis in turkeys. Avian Dis 1993; 37:1-5.

Nivas SC, Peterson AC, York MD, Pomeroy BS, Jacobson LD, Jordan KA. Epizootiological investigations of colibacillosis in turkeys. Avian Dis 1977; 21:514-30.

Orskov I, Orskov F, Jann B, Jann K. Serology, Chemistry, and Genetics of O and K antigens of *Escherichia coli*. Bacteriol. Rev.1977; 41:667-710.

Oswald E, Marches E, Morabito S, Caprioli A. Insertion and stability of the LEE pathogenicity island in EHEC and EPEC: analysis of tRNA hot spots as a useful tool in epidemiological studies. Nature 2000; 6:64-7.

Otto BR, van Dooren SJ, Nuijens JH, Luirink J, Oudega B. Characterization of a hemoglobin protease secreted by the pathogenic *Escherichia coli* strain EB1. J. Exp. Med. 1998; 188:1091-103.

Owings W. Colibacillosis is big health problem for Iowa turkey growers. Poultry Times. 1995; 8:28.

Palumbi SR. Humans as the World's Greatest Evolutionary Force. Science 2001; 7:1786-90.

Parreira VR, Yano T. Cytotoxin produced by *Escherichia coli* isolated from chickens with swollen head syndrome (SHS). Vet Microbiol 1998;62:111-19.

Parveen S, Hodge NC, Stall RE, Farrah SR, Tamplin ML. Phenotypic and genotypic characterization of human and nonhuman *Escherichia coli*. Water Res 2001; 35:379-86.

Peighambari SM, Gyles CL. Construction and characterization of avian *Escherichia coli cya crp* mutants. Avian Dis 1998; 42:698-710.

Pezzlo, M. Aerobic Bacteriology. In: Clinical Microbiology Procedures Handbook (Volume 1). Isenberg HD, ed. American Society of Microbiology, Washington, DC. 1992; pp. 1-34.

Pfaff-McDonough SJ, Horne SM, Giddings CW, Ebert JO, Doetkott C, Smith MH, Nolan LK. Complement resistance-related traits among *Escherichia coli* isolates from apparently healthy birds and birds with colibacillosis. Avian Dis 2000; 44:23-33.

PhPlate. Worldwide Web: http://www.phplate.se/. Accessed October 6, 2001.

Pierson FW, Barta VD, Boyd D, Thompson WS. Exposure to multiple infectious agents and the development of colibacillosis in turkeys. J Applied Poultry Res 1996; 5:347-7.

Pierson FW, Larsen CT, Domermuth CH. The production of colibacillosis in turkeys following sequential exposure to Newcastle disease virus or *Bordetella avium*, avirulent hemorrhagic enteritis virus, and *Escherichia coli*. Avian Dis 1996; 40:837-40.

Pomeroy BS. An overview: colibacillosis in turkeys. Poultry Digest. (Mount Morris, Ill. Watt Publishing Company) 1984; 506:138-40.

Poss PE. Turkey industry strategies for control of respiratory and enteric diseases. Poult Sci 1998; 77:1181-5.

Pourbakhsh SA, Boulianne M, Martineau-Doize B, Dozois CM, Desautels C, Fairbrother JM. Dynamics of *Escherichia coli* infection in experimentally inoculated chickens. Avian Dis 1997; 41:221-33.

Pourbakhsh SA, Dho-Moulin M, Bree A, Desautels C, Martineau-Doize B, Fairbrother JM. Localization of the in vivo expression of P and F1 fimbriae in chickens experimentally inoculated with pathogenic *Escherichia coli*. Microb Pathog 1997; 22:331-341.

Provence D L, Curtiss III R. Isolation and characterization of a gene involved in hemagglutination by an avian pathogenic *Escherichia coli* strain. Infect Immun 1994; 62:1369-80.

Ramirez Santoyo RM, Moreno Sala A, Almanza Marquez Y. Avian *Escherichia coli* virulence factors associated with colisepticemia in broiler chickens. Rev Argent Microbiol 2001; 33:52-57.

Reid SD, Herbelin CJ, Bumbaugh AC, Selander RK, Whittam TS. Parallel evolution of virulence in pathogenic *Escherichia coli*. Mol Biol Evol 1999; 16:12-22.

Reingold J, Starr N, Maurer J, Lee MD. Identification of a new *Escherichia coli* She haemolysin homolog in avian *E. coli*. Vet Microbiol 1999; 66:125-34.

Roland K, Curtiss III R, Sizemore D. Construction and evaluation of a delta *cya* delta *crp* *Salmonella* typhimurium strain expressing avian pathogenic *Escherichia coli* O78 LPS as a vaccine to prevent airsacculitis in chickens. Avian Dis 1999; 43:429-41.

Rolfe DL, Riemann HP, Farver TB, Himathongkham S. Drag swab efficiency factors when sampling chicken manure. Avian Dis 2000; 44:668-75.

Salmon SA, Watts JL. Minimum inhibitory concentration determinations for various antimicrobial agents against 1570 bacterial isolates from turkey poults. Avian Dis 2000; 44:85-98.

Singer RS, Johnson WO, Jeffrey JS, Chin RP, Carpenter TE, Atwill ER, Hirsh DC. A statistical model for assessing sample size for bacterial colony selection: a case study of *Escherichia coli* and avian cellulitis. J Vet Diagn Invest 2000; 12:118-25.

Smith HW, Cook JK, Parsell ZE. The experimental infection of chickens with mixtures of infectious bronchitis virus and *Escherichia coli*. J Gen Virol 1985; 66:777-86.

Smith KE, Besser JM, Hedberg CW, Leano FT, Bender JB, Wicklund JH, Johnson BP, Moore KA, Osterholm MT. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. N Engl J Med 1999; 340:1525-32.

Sneath PHA, Sokal RR. Numerical Taxonomy. WH Freeman and Company, San Francisco, CA.1973; pp. 230-4.

Sojka WJ, Carnaghan RBA. *Escherichia coli* infection in poultry. Res Vet Sci 1961; 2:340-51.

Spika JS, Waterman SH, Hoo GW, St Louis ME, Pacer RE, James SM, Bissett ML, Mayer LW, Chiu JY, Hall B. Chloramphenicol-resistant *Salmonella newport* traced through hamburger to dairy farms. A major persisting source of human salmonellosis in California. N Engl J Med 1987; 316:565-70.

Tablante NL, Brunet PY, Odor EM, Salem M, Harter-Dennis JM, Hueston WD. Risk factors associated with early respiratory disease complex in broiler chickens. Avian Dis 1999; 43:424-8.

Tierney L. Markov chains for exploring posterior distributions. The Annals of Statistics, 1994; 22:1701-1762.

Tollefson L, Angulo F, Fedorka-Cray P. National surveillance for antibiotic resistance in zoonotic enteric pathogens. Veterinary Clinics of North America: Food Animal Practice 1998; 14:141-50.

Turtura GC, Massa S, Ghazvinizadeh H. Antibiotic resistance among coliform bacteria isolated from carcasses of commercially slaughtered chickens. Int J Food Microbiol 1990;11:351-4.

Tsukamoto T. PCR method for detection of K1 antigen and serotypes of *Escherichia coli* isolated from extraintestinal infection. J Jap Assoc Infect Dis 1997; 71:125-9.

Valvano MA. Diphenylamine increases cloacin DF13 sensitivity in avian septicemic strains of *Escherichia coli*. Vet Microbiol 1992; 32:149-61.

Van de Zande S, Nauwynck H, Pensaert M. The clinical, pathological and microbiological outcome of an *Escherichia coli* O2:K1 infection in avian pneumovirus infected turkeys. Vet Microbiol 2001; 81:353-65.

Van den Bogaard AE, London N, Driessen C, Stobberingh EE. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. J Antimicrob Chemother 2001; 47:763-71.

Van den Bosch JF, Hendriks JH, Gladigau I, Willems HM, Storm PK, de Graaf FK. Identification of F11 fimbriae on chicken *Escherichia coli* strains. Infect Immun 1993; 61:800-6.

Van den Hurk J, Allan BJ, Riddell C, Watts T, Potter AA. Effect of infection with hemorrhagic enteritis virus on susceptibility of turkeys to *Escherichia coli*. Avian Dis 1994; 38:708-1650.

Vidotto MC, Cacao JM, Goes CR, Santos DS. Plasmid coding for aerobactin production and drug resistance is involved in virulence of *Escherichia coli* avian strains. Braz J Med Biol Res 1991; 24:677-85.

Vimr ER. Map position and genomic organization of the *kps* cluster for polysialic acid synthesis in *Escherichia coli* K1. J Bacteriol 1991; 173:1335-8.

Vokes SA, Reeves SA, Torres AG, Payne SM. The aerobactin iron transport system genes in *Shigella flexneri* are present within a pathogenicity island. Mol Microbiol 1999; 33:63-73.

Weebadda WK, Hoover GJ, Hunter DB, Hayes MA. Avian air sac and plasma proteins that bind surface polysaccharides of *Escherichia coli* O2. Comp Biochem Physiol B Biochem Mol Biol 2001; 130:299-312.

Weinack OM, Snoeyenbos GH, Smyser CF, Soerjadi AS. Competitive exclusion of intestinal colonization of *Escherichia coli* in chicks. Avian Dis 1981; 25:696-705.

White, DG, Dho-Moulin M, Wilson RA, Whittam TS. Clonal relationships and variation in virulence among *Escherichia coli* strains of avian origin. Microb Pathog 1993; 14:399-409.

White DG, Piddock LJ, Maurer JJ, Zhao S, Ricci V, Thayer SG. Characterization of fluoroquinolone resistance among veterinary isolates of avian *Escherichia coli*. Antimicrob Agents Chemother 2000; 44:2897-9.

White DG, Wilson RA, Emery DA, Nagaraja KV, Whittam TS. Clonal diversity among strains of *Escherichia coli* incriminated in turkey colisepticemia. Vet. Microbiol. 1993; 34:19-34.

White DG, Zhao S, Sudler R, Ayers S, Friedman S, Chen S, McDermott PF, McDermott S, Wagner DD, Meng J. The isolation of antibiotic-resistant salmonella from retail ground meats. N Engl J Med 2001;345:1147-54.

Whittam TS, Wilson RA. Genetic relationships among pathogenic strains of avian *Escherichia coli*. Infect Immun 1988; 56:2458-66.

Winokur PL, Brueggemann A, DeSalvo DL, Hoffmann L, Apley MD, Uhlenhopp EK, Pfaller MA, Doern GV. Animal and human multidrug-resistant, cephalosporin-resistant *Salmonella* Isolates expressing a plasmid-mediated *CMY*-2 AmpC beta-lactamase. Antimicrob Agents Chemother 2000; 44:2777-83.

Wooley RE, Gibbs PS, Brown TP, Maurer JJ. Chicken embryo lethality assay for determining the virulence of avian *Escherichia coli* isolates. Avian Dis 2000; 149:318-24.

Wooley RE, Spears KR, Brown J, Nolan LK, Dekich MA. Characteristics of conjugative R-plasmids from pathogenic avian *Escherichia coli*. Avian Dis 1992; 36:348-52.

Wray C, Gnanou JC. Antibiotic resistance monitoring in bacteria of animal origin: analysis of national monitoring programmes, International Journal of Antimicrobial Agents 2000; 14:291-4.

York MK, Baron EJ, Clarridge JE, Thomson RB, Weinstein MP. Multilaboratory validation of rapid spot tests for identification of *Escherichia coli*. J Clin Microbiol 2000; 38:3394-8.

Zhao S, White DG, McDermott PF, Friedman S, English L, Ayers S, Meng J, Maurer JJ, Holland R, Walker RD. Identification and expression of cephamycinase bla*cmy* genes in *Escherichia coli* and *Salmonella* isolated from food animals and ground meats. Antimicrobial and Chemotherapy, December 2001 (In Press).

APPENDIX A
Risk Factor Analyses Relating Age of Flock and Antimicrobial Usage to Resistance

Materials and methods

Regression analyses were performed (SAS, Proc Reg) to relate the age of the flock in weeks to mean MIC values of fecal *E. coli* isolates for each flock. Analyses were also performed (SAS, Proc GLM) to relate reported antimicrobial usage to mean MIC values of fecal *E. coli* isolates for each flock. In the latter analyses, we estimated the power of our data to detect an association when it occurred, given the sample size and effect at a 0.05 significance level. The lowest number of flocks needed to obtain a p-value of 0.05 was also calculated, given the sample size and effect of MIC related to antimicrobial usage. The calculations were conducted using a SAS macro constructed for use with SAS, Proc GLM.

Results

Age and resistance

There was a suggestion of a gradient of declining resistance to the quinolone, nalidixic acid, and the fluoroquinolone, ciprofloxacin, in brooder flocks with increasing age of flock in weeks (Table 1). In finisher flocks, gentamicin resistance appeared declined with increasing age.

Antimicrobial usage and resistance

The data on 12 brooder flocks provided insufficient power to associate antimicrobial usage was associated with resistance in fecal *E. coli*; however, there was a suggestion of increasing resistance to ciprofloxacin (and perhaps nalidixic acid) with use of enrofloxacin (Table 2). Borderline significant associations were also seen between usage of enrofloxacin and resistance to ciprofloxacin and nalidixic acid in finisher flocks, although the magnitude of resistance was less than in brooder flocks. Similarly, there were possible associations between penicillin usage and resistance to ampicillin and perhaps to amoxicillin/clavulinic acid, ceftiofur, and cephalothin.

Table 1. Results of regression analyses relating geometric mean MIC values (log 2) and age of flock in weeks (independent variable) for 12 brooder flocks (six weeks old or younger) and 20 finisher flocks (10 weeks of age or greater).

	Brooders flocks		Finisher flocks	
	Slope	p-value	Slope	p-value[a]
Nalidixic acid	–0.86	0.05	–0.06	0.83
Ciprofloxacin	–0.62	0.07	–0.01	0.98
Cephalothin	0.23	0.09	0.07	0.51
Amoxicillin/ Clavulinic acid	0.23	0.14	–0.02	0.77
Ampicillin	0.35	0.22	–0.02	0.91
Gentamicin	0.20	0.57	–0.39	0.03

[a] H_o: slope ß = 0. No other notable results (i.e., slope greater than |0.15| or p-value < 0.25 were observed. Other regression analyses were performed that related median MIC to age in weeks for the following antimicrobial drugs: amikacin, apramycin, kanamycin, streptomycin, chloramphenicol, florfenicol, ceftiofur, ceftriaxone, sulfamethoxazole, trimethoprim/sulfamethoxazole, and tetracycline.

Table 2. Results of generalized linear model analyses relating geometric mean MIC (log 2) of fecal *E. coli* to antimicrobial usage in turkey flocks.

	Brooder flocks				Finisher flocks			
Drug	No. flocks	Mean MIC	p-value	Power (LN)	No. flocks	Mean MIC	p-value	Power (LN)
Enrofloxacin								
Ciprofloxacin								
No	10	0.04	0.13	0.17 (20)	8	0.03	0.12	0.21 (31)
Yes	2	0.15			12	0.05		
Nalidixic acid								
No	10	16	0.21	0.09 (29)	8	9.2	0.16	0.15 (38)
Yes	2	69[a]			12	17		
Penicillin								
Ampicillin								
No	7	13	0.10	0.19 (18)	10	16[b]	0.38	0.05 (96)
Yes	5	32 [a]			10	12		
Amoxicillin/Penicillin								
Clavulinic acid								
No	7	4.3	0.17	0.20 (26)	10	4.9	0.73	0.05 (558)
Yes	5	6.5			10	4.6		
Penicillin								
Ceftiofur								
No	7	0.51	0.17	0.25 (26)	10	0.51	0.29	0.05 [c]
Yes	5	0.57			10	0.52		
Cetriaxone								
No	7	0.25	0.26	0.07 (34)	10	0.28	0.40	0.05 (111)
Yes	5	0.28			10	0.28		
Cephalothin								
No	7	9[b]	0.45	0.10 (27)	10	10.6[b]	0.80	0.05 (853)
Yes	5	13[b]			10	9.8[b]		

NOTE: LN = lowest number of flocks to obtain a p-value <0.05, assuming fixed effect between antimicrobial usage and geometric mean MIC.

[a]MIC in resistant range.

[b]MIC in intermediate susceptibility range.

[c] Geometric mean MICs were similar and it was therefore not possible to determine the lowest number of flocks to obtain a p-value <0.05, assuming fixed effect between antimicrobial usage and geometric mean MIC with the power macro software.

APPENDIX B
Survey Frequencies

The following data were obtained through interviews conducted with field services personnel, observations made during farm visits, and analyses of specimens collected from flocks. A total of 20 turkey flocks with hens between 10 and 14 weeks of age and 12 turkey flocks with poults less than 7 weeks of age were sampled (approximately 15% of all flocks in the integrated operation during the sampling period. Fieldwork was conducted during six weeks in the summer of 1999 during a period when daily high temperatures exceeded 90^{o}F and drought conditions prevailed.

How old are the birds in this brooder unit? ___ WEEKS

BRDRAGW	Frequency	Percent	Cumulative frequency	Cumulative percent
0.5	1	8.33	1	8.33
1	1	8.33	2	16.67
2	4	33.33	6	50.00
3	4	33.33	10	83.33
4	1	8.33	11	91.67
6	1	8.33	12	100.00

Number of birds placed in this brooder unit _____

NUMPLC	Frequency	Percent	Cumulative Frequency	Cumulative Percent
10-11000	1	8.33	1	8.33
11-12000	10	83.33	11	91.66
25964	1	8.33	12	100.00

Two week poult mortality in this brooder unit _____

TWKMTBR	Frequency	Percent	Cumulative frequency	Cumulative percent
150-200	3	30.00	3	30.00
236	2	20.00	5	50.00
300-350	2	20.00	7	70.00
490	1	10.00	8	80.00
610	1	10.00	9	90.00
1288	1	10.00	10	100.00

Two week mortality rate in this brooder unit _____

TWMRATE	Frequency	Percent	Cumulative frequency	Cumulative percent
0.01-0.02	3	30.00	3	30.00
0.02-0.03	4	40.00	7	70.00
0.04-0.05	2	20.00	9	90.00
0.052137	1	10.00	10	100.00

Total mortality to date in this brooder unit _____

TOMTBDR	Frequency	Percent	Cumulative frequency	Cumulative percent
50-100	2	16.66	2	16.66
150-200	2	16.66	4	33.32
268	1	8.33	5	41.67
300-350	3	25	8	66.67
372	1	8.33	9	75.00
516	1	8.33	10	83.33
610	1	8.33	11	91.67
1386	1	8.33	12	100.00

Mortality rate till now in this brooder unit _____

BMRTNOW	Frequency	Percent	Cumulative frequency	Cumulative percent
>0.01	2	16.67	2	16.67
0.01-0.02	2	16.67	4	33.67
0.02-0.03	3	25.00	7	58.33
0.03-0.04	2	16.67	9	75.00
0.044944	1	8.33	10	83.33
0.05-0.055	2	16.67	12	100.00

Have birds in the brooder unit had any disease problems?

ANDSBRD	Frequency	Percent	Cumulative frequency	Cumulative percent
1	8	66.67	8	66.67
2	4	33.33	12	100.00

If poults in this brooder have had disease problems, please describe

Symptom 1, brooder unit

BRSYMP1	Frequency	Percent	Cumulative frequency	Cumulative percent
Airsac	1	12.50	1	12.50
Enteritis	1	12.50	2	25.00
Loose	1	12.50	3	37.50
osteomyel	1	12.50	4	50.00
overheated	1	12.50	5	62.50
Poor poult	2	2.5.00	7	87.50
respiratory	1	12.50	8	100.00

Week of onset of symptom 1, brooder unit

ONSY1BR	Frequency	Percent	Cumulative frequency	Cumulative percent
0	1	16.67	1	16.67
1	2	33.33	3	50.00
2	2	33.33	5	83.33
6	1	16.67	6	100.00

Brooder flock vaccinated against Bordatella?

BRDVBRD	Frequency	Percent	Cumulative frequency	Cumulative percent
YES	1	8.33	1	8.33
NO	11	91.67	12	100.00

Brooder flock vaccinated against Newcastle Disease Virus?

NEWVBR	Frequency	Percent	Cumulative frequency	Cumulative percent
NO	12	100.00	12	100.00

Brooder flock vaccinated against Hemorrhagic Enteritis virus?

HEVCBR	Frequency	Percent	Cumulative frequency	Cumulative percent
YES	4	33.33	4	33.33
NO	8	66.67	12	100.00

Age brooder flock vaccinated against Hemorrhagic Enteritis virus?

HEVAGBR	Frequency	Percent	Cumulative frequency	Cumulative percent
3 weeks	3	75.00	3	75.00
4 weeks	1	25.00	4	100.00

How old are the hens in the finisher unit? _____ WEEKS

FINAGE	Frequency	Percent	Cumulative frequency	Cumulative percent
10	1	5.00	1	5.00
11	5	25.00	6	30.00
11.5	1	5.00	7	35.00
12	7	35.00	14	70.00
13	3	15.00	17	85.00
14	3	15.00	20	100.00

Number of birds placed (finisher flock)

FINPLC	Frequency	Percent	Cumulative frequency	Cumulative percent
10-11000	1	5.00	1	5.00
11-12000	17	85.00	18	90.00
15675	1	5.00	19	95.00
25990	1	5.00	20	100.00

Two week mortality (finisher flock)

TWKMTFN	Frequency	Percent	Cumulative frequency	Cumulative percent
<100	1	5.00	1	5.00
100-200	7	35.00	8	40.00
200-300	5	25.00	13	65.00
300-400+	4	20.00	17	85.00
739	1	5.00	18	90.00
1291	1	5.00	19	95.00
1662	1	5.00	20	100.00

Mortality since move to finisher (finisher flock)

FINMTMV	Frequency	Percent	Cumulative frequency	Cumulative percent
>100	5	25	5	25.00
100-200	8	40	13	65.00
259	1	5	14	70.00
390	1	5	15	75.00
400-500	2	10	17	85.00
600	1	5.26	17	89.47
816	1	5.26	18	94.74
1600	1	5.26	19	100.00

TOMTFIN	Frequency	Percent	Cumulative frequency	Cumulative percent
400-600	10	50	10	50.00
600-1000	5	25	15	75.00
1000-2000	3	15	18	90.00
2000-3000	2	10	20	100.00

Have birds in the finisher unit had any disease problems? If YES, please describe

	Disease/Syndrome	Age at onset	Treatment
A.	_____	_____WEEKS	_____
B.	_____	_____WEEKS	_____

Have birds in the finisher unit had any disease problems?

ANDSFIN	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	18	94.74	18	94.74
No	1	5.26	19	100.00

Frequency Missing = 1

Symptom 1, finisher unit

FINSYM1	Frequency	Percent	Cumulative frequency	Cumulative percent
E. coli	3	15.00	3	15.00
Staph infection	2	10.00	5	25.00
Air saccul	3	15.00	8	40.00
bordatella	1	5.00	9	45.00
enteritis	5	25.00	14	70.00
Inf yolks	1	5.00	15	75.00
loose	2	10.00	17	85.00
overheated	1	5.00	18	90.00
sinusitis	1	5.00	19	95.00
Anorexia	1	5.00	20	100.00

Week of onset, symptom 1, finisher unit

ONSY1FN	Frequency	Percent	Cumulative frequency	Cumulative percent
0	2	10.53	2	10.53
1	4	21.05	6	31.58
2	2	10.53	8	42.11
3	4	21.05	12	63.16
6	3	15.79	15	78.95
7	1	5.26	16	84.21
8	1	5.26	17	89.47
9	1	5.26	18	94.74
11	1	5.26	19	100.00

Frequency Missing = 1

Symptom 2, finisher unit

FNSYM2	Frequency	Percent
E coli	2	18.18
air sacc	5	25.00
Enteritis	1	9.09
Poor poult	2	10.00
Pseudomonas	1	9.09

Frequency Missing = 9

Week of onset, symptom 2, finisher unit

ONSY2FN	Frequency	Percent	Cumulative frequency	Cumulative percent
0	1	9.09	1	9.09
1	1	9.09	2	18.18
2	1	9.09	3	27.27
3	2	18.18	5	45.45
4	1	9.09	6	54.55
5	2	18.18	8	72.73
7	1	9.09	9	81.82
8	2	18.18	11	100.00

Frequency Missing = 9

Finisher flock vaccinated against Bordatella?

BORDVFN	Frequency	Percent	Cumulative frequency	Cumulative Percent
Yes	2	10.00	2	10.00
No	18	90.00	20	100.00

Age finisher flock vaccinated against Bordatella?

BORDAGF	Frequency	Percent	Cumulative frequency	Cumulative percent
0 weeks	1	50.00	1	50.00
3 weeks	1	50.00	2	100.00

Frequency Missing = 18

Finisher flock vaccinated against Newcastle Virus?

NEWVCFN	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	3	15.00	3	15.00
No	17	85.00	20	100.00

Age finisher flock vaccinated against Newcastle virus?

NWVAGF	Frequency	Percent	Cumulative frequency	Cumulative percent
2 weeks	3	100.00	3	100.00

Frequency Missing = 17

Finisher flock vaccinated against Hemorrhagic enteritis virus?

HEVCFN	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	18	90.00	18	90.00
No	2	10.00	20	100.00

Age finisher flock vaccinated against Hemorrhagic enteritis virus?

HEVAGF	Frequency	Percent
3	14	73.68
3.5	2	10.53
4	2	10.53

Frequency Missing = 2

Was any antibiotic ever used in brooder flock?

ABXRXBR	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	4	33.33	4	33.33
No	8	66.67	12	100.00

Was any antibiotic treatment ever used in finisher flock?

ABXRXFN	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	2	10.00	2	10.00
No	18	90.00	20	100.00

Was any tetracycline class drug ever used in brooder flock?

COTET	Frequency	Percent	Cumulative frequency	Cumulative percent
No	8	66.67	8	66.67
Yes	4	33.33	12	100.00

Was any tetracycline class drug ever used in finisher flock?

COTEFNM	Frequency	Percent	Cumulative frequency	Cumulative percent
No	9	45.00	9	45.00
Once	10	50.00	19	95.00
Twice	1	5.00	20	100.00

Was chlortetracycline ever used in brooder?

CLTEBRD	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	1	8.33	1	8.33
No	11	91.67	11	100

Is chlortetracycline currently used in finisher flock?

CLTFNOW	Frequency	Percent	Cumulative frequency	Cumulative percent
No	20	100.00	20	100.00

Total treatments chlortetracycline in finisher flock? _____

CLTFNUM	Frequency	Percent	Cumulative frequency	Cumulative percent
0	16	80.00	16	80.00
1	4	20.00	20	100.00

Was copper sulfate ever used in brooder flock ?

CUSOBRD	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	1	8.33	1	8.33
No	11	91.67	12	100.00

Is copper sulfate currently used in finisher flock?

CUSFNOW	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	2	10.00	2	10.00
No	18	90.00	20	100.00

Total treatments copper sulfate in finisher flock? ____

CUSFNUM	Frequency	Percent	Cumulative frequency	Cumulative percent
0	12	63.16	12	63.16
1	1	5.26	13	68.42
2	2	10.53	15	78.95
3	2	10.53	17	89.47
4	1	5.26	18	94.74
10	1	5.26	19	100.00

Frequency Missing = 1

Was enrofloxacin ever used in brooder flock?

ENROBRD	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	2	16.67	2	16.67
No	10	83.33	12	100.00

Is enrofloxacin currently used in finisher flock?

ENRONOW	Frequency	Percent	Cumulative frequency	Cumulative percent
No	20	100.00	20	100.00

Total treatments with enrofloxacin in finisher flock? _____

ENROFNM	Frequency	Percent	Cumulative frequency	Cumulative percent
0	12	60.00	12	60.00
1	7	35.00	19	95.00
2	1	5.00	20	100.00

Was erythromicin ever used in brooder flock?

ERYTBRD	Frequency	Percent	Cumulative frequency	Cumulative percent
No	12	100.00	12	100.00

Is erythromicin currently used in finisher flock?

ERYFNW	Frequency	Percent	Cumulative frequency	Cumulative percent
No	20	100.00	20	100.00

Total treatments with erythromicin in finisher flock ____

ERYFNM	Frequency	Percent	Cumulative frequency	Cumulative percent
0	19	95.00	19	95.00
1	1	5.00	20	100.00

Was neomycin ever used in brooder flock?

NEOBRD	Frequency	Percent	Cumulative frequency	Cumulative percent
No	12	100.00	12	100.00

Is neomycin currently used in finisher flock?

NEOFNW	Frequency	Percent	Cumulative frequency	Cumulative percent
No	20	100.00	20	100.00

Total treatments with neomycin in finisher flock? _____

NEOFNM	Frequency	Percent	Cumulative frequency	Cumulative percent
0	20	100.00	20	100.00

Was oxytetracycline ever used in brooder flock?

OXYTBRD	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	1	8.33	1	8.33
No	11	91.67	12	100.00

Is oxytetracycline currently used in finisher flock?

OXYFNW	Frequency	Percent	Cumulative frequency	Cumulative percent
No	20	100.00	20	100.00

Total treatments with oxytetracycline in finisher flock? _____

OXYTFNM	Frequency	Percent	Cumulative frequency	Cumulative percent
0	12	60.00	12	60.00
1	8	40.00	20	100.00

Was penicillin ever used in brooder flock?

PENIBRD	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	4	33.33	4	33.33
No	8	66.67	12	100.00

Is penicillin currently used in finisher flock?

PENFNOW	Frequency	Percent	Cumulative frequency	Cumulative percent
No	20	100.00	20	100.00

Total treatments with penicillin in finisher flock? _____

PENFNUM	Frequency	Percent	Cumulative frequency	Cumulative percent
0	10	50.00	10	50.00
1	8	40.00	18	90.00
2	2	10.00	20	100.00

Was sulfamethazine ever used in brooder flock?

SULMBRD	Frequency	Percent	Cumulative frequency	Cumulative percent
No	12	100.00	12	100.00

Is sulfamethazine currently used in finisher flock?

SLFMFNW	Frequency	Percent	Cumulative frequency	Cumulative percent
No	20	100.00	20	100.00

Total treatments with sulfamethazine in finisher flock _____

SLFMFNM	Frequency	Percent	Cumulative frequency	Cumulative percent
0	18	90.00	18	90.00
1	2	10.00	20	100.00

Ws sulfaquinoxalin ever used in brooder flock?

SULFBRD	Frequency	Percent	Cumulative frequency	Cumulative percent
No	12	100.00	12	100.00

Is sulfaquinoxalin currently used in finisher flock?

SULQFNW	Frequency	Percent	Cumulative frequency	Cumulative percent
No	20	100.00	20	100.00

Total treatments with sulfaquinoxalin in finisher flock? _____

SULQFNM	Frequency	Percent	Cumulative frequency	Cumulative percent
0	19	95.00	19	95.00
2	1	5.00	20	100.00

Was any sulfa drug ever used in brooder flock?

SULFABRD	Frequency	Percent	Cumulative frequency	Cumulative percent
0	12	100	12	100

Total treatments with any sulfa drug in finisher flock? _____

SULFSFN	Frequency	Percent	Cumulative frequency	Cumulative percent
0	18	90.00	18	90.00
1	1	5.00	19	95.00
2	1	5.00	20	100.00

Was tetracycline ever used in brooder flock?

TETRBRD	Frequency	Percent	Cumulative frequency	Cumulative percent
No	12	100.00	12	100.00

Is tetracycline currently used in finisher flock?

TETRNOW	Frequency	Percent	Cumulative frequency	Cumulative percent
No	20	100.00	20	100.00

Total treatments with tetracycline in finisher flock ? _____

TETRFNM	Frequency	Percent	Cumulative frequency	Cumulative percent
0	20	100.00	20	100.00

Was tylan ever used in brooder flock?

TYLBRD	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	3	25.00	3	25.00
No	9	75.00	12	100.00

Is tylan currently used in finisher flock?

TYLNOW	Frequency	Percent	Cumulative frequency	Cumulative percent
No	20	100.00	20	100.00

Total treatments with tylan in finisher flock? _____

TYLNUM	Frequency	Percent	Cumulative frequency	Cumulative percent
0	16	80.00	16	80.00
1	3	15.00	19	95.00
2	1	5.00	20	100.00

Growers questionnaire

In what year was this house built? 19 __ __

HOUSYR	Frequency	Percent	Cumulative frequency	Cumulative percent
1977	1	5.00	1	5.00
1979	2	10.00	3	15.00
1983	4	20.00	7	35.00
1984	1	5.00	8	40.00
1986	1	5.00	9	45.00
1987	3	15.00	12	60.00
1988	1	5.00	13	65.00
1991	3	15.00	16	80.00
1992	1	5.00	17	85.00
1993	1	5.00	18	90.00
1995	2	10.00	20	100.00

Turkey water source Municipal Spring Well Other

WATRSRC	Frequency	Percent	Cumulative frequency	Cumulative percent
Well	20	100.00	20	100.00

How often do you clean and disinfect drinkers? Standardized to ____/Year

CLNDRNK	Frequency	Percent	Cumulative frequency	Cumulative percent
2	1	5.26	1	5.26
3	3	15.79	4	21.05
12	2	10.53	6	31.58
24	1	5.26	7	36.84
52	3	15.79	10	52.63
104	4	21.05	14	73.68
156	3	15.79	17	89.47
250	1	5.26	18	94.74
256	1	5.26	19	100.00

Frequency Missing = 1

What type of water sanitation do you use?
Hypochlorite Chlorine dioxide Ozone Peroxide Other (Specify)_____ None

WATRCHM	Frequency	Percent	Cumulative frequency	Cumulative percent
Hypochlorite	18	90.00	18	90.00
ClO_2	1	5.00	19	95.00
None	1	5.00	20	100.00

How often are water lines in the finisher unit descaled and sanitized?
After each flock Every second flock Less often

DESCALE	Frequency	Percent	Cumulative frequency	Cumulative percent
After each	11	55.00	11	55.00
Every second	7	35.00	18	90.00
Less often	2	10.00	20	100.00

How often are dead birds picked up? Twice a day Every day Every other day Less often

DEADPIK	Frequency	Percent	Cumulative frequency	Cumulative percent
1	13	65.00	13	65.00
2	7	35.00	20	100.00

How do you dispose of dead birds? Compost Bury Render Incinerate Other _____

DEADDSP	Frequency	Percent	Cumulative frequency	Cumulative percent
Compost	15	75.00	15	75.00
Render	4	20.00	19	95.00
Incinerate	1	5.00	20	100.00

How do you dispose of litter? Compost Spread on fields (Nearby or remote) Other

LITTDIS	Frequency	Percent	Cumulative frequency	Cumulative percent
Compost	1	5.00	1	5.00
Spread on field	19	95.00	20	100.00

Is the field near or far from the turkey house?

FLDNRFR	Frequency	Percent	Cumulative frequency	Cumulative percent
Near	11	61.11	11	61.11
Far	5	27.78	16	88.89
No answer	2	11.11	18	100.00

Frequency Missing = 2

When was the feed tank last cleaned and sanitized? ___ Month ___ Year Never

FEEDTNK	Frequency	Percent	Cumulative frequency
Before 1990	3	32.86	3
1991	2	28.57	2
1996	1	14.29	3
1998	1	14.29	4

Frequency Missing = 13

How long was the finisher empty between this flock and the last? _____ Days

FINEMP	Frequency	Percent	Cumulative frequency	Cumulative percent
7	2	10.00	2	10.00
10-19	14	70.00	16	80.00
20-28	4	20.00	20	100.00

When was the last time all houses on the farm were empty at the same time?
___ Month ___ Year Never

LASTDPP	Frequency	Percent	Cumulative frequency
1999	3	23.1	3
1998	5	38.5	8
1997	1	7.69	9
1996	1	7.69	10
1995	1	7.69	11
1991	2	15.38	13

Frequency Missing = 7

Do you work with poultry at other locations?

OTHPLWK	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	2	10.53	2	10.53
No	17	89.47	19	100.00

Frequency Missing = 1

Do you work with turkeys at other locations?

TURKWK	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	2	66.67	2	66.67
No	1	33.33	3	100.00

Frequency Missing = 17

Specify location of turkeys

TRKWKLOC	Frequency	Percent	Cumulative frequency	Cumulative percent
Farm	2	100	2	100

Do you work with broilers at other locations?

BRLRWK	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	1	100	1	100

Specify location of broilers

BRLRWLC	Frequency	Percent	Cumulative frequency	Cumulative percent
Farm	1	100.00	1	100.00

Do your employees work with poultry at other locations?

EMPPLWK	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	3	18.75	3	18.75
No	13	81.25	16	100.00

Frequency Missing = 4

Do your employees work with turkeys at other locations?

EMPTRK	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	3	100.00	3	100.00

Frequency Missing = 17

Where do your employees work with turkeys at other locations?

EMPTKLO	Frequency	Percent	Cumulative frequency	Cumulative percent
Farm	3	100	3	100

Do your employees work with broilers at other locations?

EMPBR	Frequency	Percent	Cumulative frequency	Cumulative percent
No	2	100.00	2	100.00

Frequency Missing = 18

Do you work with other livestock?

OTHLVST	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	14	70.00	14	70.00
No	6	30.00	20	100.00

Do you work with dairy cattle?

OTHDRY	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	7	43.75	7	43.75
No	9	56.25	16	100.00

Frequency Missing = 4

Do you work with beef cattle?

OTBEEF	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	7	43.75	7	43.75
No	9	56.25	16	100.00

Do you work with horses?

OTHRS	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	2	12.50	2	12.50
No	14	87.50	16	100.00

Do you work with sheep?

OTSHEE	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	3	18.75	3	18.75
No	13	81.25	16	100.00

Do your employees work with other livestock?

EMPLVST	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	6	37.50	6	37.50
No	10	62.50	16	100.00

Frequency Missing = 4

Do your employees work with dairy cattle?

EMPDRY	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	4	44.44	4	44.44
No	5	55.56	9	100.00

Frequency Missing = 11

Do your employees work with beef cattle?

EMPBEEF	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	2	22.22	2	22.22
No	7	77.78	9	100.00

Frequency Missing = 11

Do your employees work with sheep?

EMPSHEE	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	2	22.22	2	22.22
No	7	77.78	9	100.00

Frequency Missing = 11

Dairy cattle within half mile of turkey house?

DRYHM	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	8	40.00	8	40.00
No	12	60.00	20	100.00

Beef cattle within half mile of turkey house?

BEEFHM	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	10	52.63	10	52.63
No	9	47.37	19	100.00

Frequency Missing = 1

Horses within half mile of turkey house?

HRSHM	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	4	22.22	4	22.22
No	14	77.78	18	100.00

Frequency Missing = 2

Sheep within half mile of turkey house?

SHEEHM	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	3	16.67	3	16.67
No	15	83.33	18	100.00

Frequency Missing = 2

Broiler chickens within half mile of turkey house?

BRLRHM	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	3	16.67	3	16.67
No	15	83.33	18	100.00

Frequency Missing = 2

Other livestock within half mile of turkey house?

OTHERHM	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	8	42.11	8	42.11
No	11	57.89	19	100.00

Frequency Missing = 1

If other livestock within half mile of turkey house, specify

OLVHMSP	Frequency
Turkeys	4
Pullets	2
Hogs	1
Mule	1
None	1

Frequency Missing = 11

How often is the dip pan solution changed? Standardized ____/Year

DIPFRYR	Frequency	Percent	Cumulative frequency	Cumulative percent
12	1	5.56	1	5.56
52	5	27.78	6	33.33
104	3	16.67	9	50.00
156	5	27.78	14	77.78
208	1	5.56	15	83.33
260	1	5.56	16	88.89
365	2	11.11	18	100.00

Frequency Missing = 2

How often are rodent baits checked and replenished?
____/WEEK _____/Month ____/Year

BAITFRQ	Frequency
2/month	2
1/month	5
6/year	2
5/year	2
1/year	1
never	3

Frequency Missing = 6

Were feed pans cleaned and disinfected before hens were placed in finisher unit?

FEEDCLN	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	14	70.00	14	70.00
No	6	30.00	20	100.00

Are dogs and cats permitted within 100 feet of the turkey house?

CATDOG	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	10	50.00	10	50.00
No	10	50.00	20	100.00

Do you wear boots that are used only for work on this farm?

FRMBOOT	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	10	50.00	10	50.00
No	10	50.00	20	100.00

Do you wear boots that are used only for work in this house?

EMPFBT	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	8	53.33	8	53.33
No	7	46.67	15	100.00

Frequency Missing = 5

Do your employees wear boots that are used only for work on this farm?

HSBOOT	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	6	30.00	6	30.00
No	14	70.00	20	100.00

Do your employees wear boots that are used only for work in this house?

EMPHSBT	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	4	25.00	4	25.00
No	11	68.75	15	93.75
Unknown	1	6.25	16	100.00

Frequency Missing = 4

Do you wear coveralls that are used only for work on this farm?

COVERFM	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	2	10.00	2	10.00
No	18	90.00	20	100.00

Do you wear coveralls that are used only for work in this house?

COVERHS	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	1	5.00	1	5.00
No	19	95.00	20	100.00

Do your employees wear coveralls that are used only for work on this farm?

EMPCVFM	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	2	12.50	2	12.50
No	14	87.50	16	100.00

Frequency Missing = 4

Do your employees wear coveralls that are used only for work in this house?

EMPCVHS	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	1	6.25	1	6.25
No	15	93.75	16	100.00

Frequency Missing = 4

Do you wear caps that are used only for work on this farm?

CAPFR	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	2	10.00	2	10.00
No	18	90.00	20	100.00

Do you wear caps that are used only for work in this house?

CAPHS	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	1	5.00	1	5.00
No	19	95.00	20	100.00

Do your employees wear caps that are used only for work on this farm?

EMPCAPF	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	2	12.50	2	12.50
No	14	87.50	16	100.00

Frequency Missing = 4

Do your employees wear caps that are used only for work in this house?

EMPCAPH	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	1	6.25	1	6.25
No	15	93.75	16	100.00

Frequency Missing = 4

Do you wash hands between work with other livestock and work on this farm?

HANDFRM	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	13	68.42	13	68.42
No	6	31.58	19	100.00

Frequency Missing = 1

Do you wash hands between work in the brooder and finisher units?

HNDBRFN	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	5	25.00	5	25.00
No	15	75.00	20	100.00

Do employees wash hands between work in brooder / finisher unit?

EMPHNBF	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	4	25.00	4	25.00
No	11	68.75	15	93.75
Unknown	1	6.25	16	100.00

Frequency Missing = 4

Do you disinfect cleanout equipment after each use?

DISCOEQ	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	18	90.00	18	90.00
No	2	10.00	20	100.00

Do you disinfect the tires of your vehicle before entering this farm?

DISVEH	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	3	15.00	3	15.00
No	16	80.00	19	95.00
Unknown	1	5.00	20	100.00

Do your employees disinfect the tires of their vehicles before entering this farm?

EMPDISV	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	2	11.76	2	11.76
No	14	82.35	16	94.12
Unknown	1	5.88	17	100.00

Frequency Missing = 3

Do you require visitors to disinfect vehicle tires before entering this farm?

VISDISV	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	8	40.00	8	40.00
No	10	50.00	18	90.00
Unknown	2	10.00	20	100.00

Are feed or gas deliver persons permitted inside this house?

DELVRHS	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	1	5.00	1	5.00
No	19	95.00	20	100.00

In the past month how many company employees logged in ____

EMPLOG	Frequency	Percent	Cumulative frequency	Cumulative percent
5	1	5.00	1	5.00
7	1	5.00	2	10.00
8	2	10.00	4	20.00
9	1	5.00	5	25.00
10	3	15.00	8	40.00
11	2	10.00	10	50.00
12	1	5.00	11	55.00
13	3	15.00	14	70.00
14	2	10.00	16	80.00
15	2	10.00	18	90.00
17	2	10.00	20	100.00

In the past month total number of people who logged in ___

LOGNUM	Frequency	Percent	Cumulative frequency	Cumulative percent
7	2	10.00	2	10.00
8	1	5.00	3	15.00
10	1	5.00	4	20.00
11	1	5.00	5	25.00
12	2	10.00	7	35.00
13	2	10.00	9	45.00
14	1	5.00	10	50.00
15	4	20.00	14	70.00
16	3	15.00	17	85.00
17	1	5.00	18	90.00
19	2	10.00	20	100.00

How far to the nearest body of water? ____Miles

WTDSML	Frequency	Percent	Cumulative frequency	Cumulative percent
<1	15	75.00	15	75.00
1	2	10.00	17	85.00
1.5	1	5.00	18	90.00
2	2	10.00	20	100.00

Nearest body of water: Pond/lake Stream/river Other ____

WATRTYP	Frequency	Percent	Cumulative frequency	Cumulative percent
Pond	1	5.00	1	5.00
Stream	18	90.00	19	95.00
Other	1	5.00	20	100.00

Any litter spills seen within 100 ft of house?

LITSPL	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	4	20.00	4	20.00
No	16	80.00	20	100.00

Any feed spills seen within 100 ft of house?

FEEDSPL	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	1	5.00	1	5.00
No	19	95.00	20	100.00

Any other debris within 100 ft of house?

DEBRIS	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	8	40.00	8	40.00
No	12	60.00	20	100.00

Is grass within 100 ft of house trimmed?

GRASSCUT	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	8	40.00	8	40.00
No	12	60.00	20	100.00

Any other farm animals within 100 ft of house?

ANIM100	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	8	40.00	8	40.00
No	12	60.00	20	100.00

Dairy cattle within 100 ft of house?

DAIR100	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	5	27.78	5	27.78
No	13	72.22	18	100.00

Frequency Missing = 2

Beef cattle within 100 ft of house?

BEEF100	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	3	16.67	3	16.67
No	15	83.33	18	100.00

Frequency Missing = 2

Horses within 100 ft of house?

HORS100	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	1	5.56	1	5.56
No	17	94.44	18	100.00

Frequency Missing = 2

Sheep within 100 ft of house?

SHEP100	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	2	11.11	2	11.11
No	16	88.89	18	100.00

Frequency Missing = 2

Goats within 100 ft of house?

GOAT100	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	18	100.00	18	100.00

Frequency Missing = 2

Broiler chickens within 100 ft of house?

BRLR100	Frequency	Percent
No	18	100.00

Frequency Missing = 2

Other livestock within 100 ft of house?

OTHR100	Frequency	Percent	Cumulative frequency	Cumulative percent
No	18	100.00	18	100.00

Frequency Missing = 2

What type of surface is the 10 ft house perimeter made of?

SUR10FT	Frequency	Percent	Cumulative frequency	Cumulative percent
Dirt	3	15.00	3	15.00
Grass	11	55.00	14	70.00
Gravel	6	30.00	20	100.00

Is there a concrete pad (stoop) outside each accessible door?

DOORPAD	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	18	90.00	18	90.00
No	2	10.00	20	100.00

Does the anteroom have a functional dip pan?

ANTDIP	Frequency	Percent	Cumulative frequency	Cumulative percent
Yes	18	90.00	18	90.00
No	2	10.00	20	100.00

What type of house is this? Open-closed (1) Closed-closed (2) Three-stage (3)

HOUSTYP	Frequency	Percent	Cumulative frequency	Cumulative percent
Open-closed	7	35.00	7	35.00
Closed-closed	10	50.00	17	85.00
Three-stage	3	15.00	20	100.00

Date of farm visit

SMPDATE	Frequency	Percent	Cumulative frequency	Cumulative percent
07/20/99	2	10.00	2	10.00
07/23/99	2	10.00	4	20.00
07/27/99	2	10.00	6	30.00
07/30/99	2	10.00	8	40.00
08/03/99	2	10.00	10	50.00
08/06/99	2	10.00	12	60.00
08/10/99	2	10.00	14	70.00
08/13/99	2	10.00	16	80.00
08/17/99	2	10.00	18	90.00
08/20/99	2	10.00	20	100.00

Number of turkey houses on premises

NUMHSES	Frequency	Percent	Cumulative frequency	Cumulative percent
1	7	35.00	7	35.00
2	13	65.00	20	100.00

Free chlorine concentration in turkey drinking water (parts per million)

FREECL	Frequency	Percent	Cumulative frequency	Cumulative percent
0	10	50.00	10	50.00
1	1	5.00	11	55.00
3	2	10.00	13	65.00
3.5	4	20.00	17	85.00
4	1	5.00	18	90.00
5	2	10.00	20	100.00

Total chlorine concentration in turkey drinking water (parts per million)

TOTCL	Frequency	Percent	Cumulative frequency	Cumulative percent
0	7	63.64	7	63.64
< 2	2	18.18	9	81.82
≥ 2	2	18.18	11	100.00

Frequency Missing = 9 (flocks with ≥ 3 ppm free chlorine)

Live beetle count in one liter of litter taken from under feeders throughout finisher room

BEETLE	Frequency	Percent	Cumulative frequency	Cumulative percent
<10	8	40.00	8	40.00
10-100	6	30.00	14	70.00
>100	6	30.00	20	100.00

Percent litter moisture of litter taken from throughout finisher room

PRCLTMST	Frequency	Percent	Cumulative frequency	Cumulative percent
<20	4	20.00	4	20.00
120-25	8	40.00	12	60.00
>25	8	40.00	20	100.00

APPENDIX C
The ß-glucouronidase Activity of Fecal *Escherichia coli* Isolates

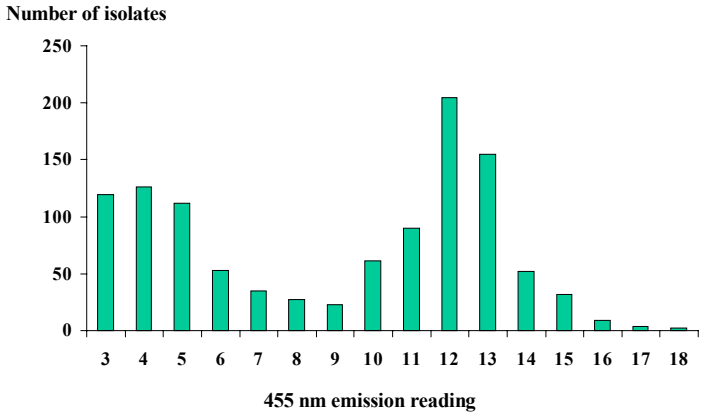
Materials and methods

To further assess ß-glucouronidase activity, sterile nonfluorescent 96 well plates containing 100 µl methylumbelliferyl- ß -D-glucouronide EC broth (Sigma-Aldrich, St. Louis, MO) were inoculated with presumptive fecal *E. coli* strains. A Cytofluor 2000 microplate reader was used to measure fluorescence [peak excitation of 365nm (UV), peak emission of 455nm (blue)]. The microplate reader made separate fluorescence readings for each well. Readings were also made of a blank control microplate, containing methylumbelliferyl- ß -D-glucouronide EC broth.

Results

The distribution of microplate readings is shown in Figure 1. Eleven percent of isolates showed no ß-glucouronidase activity (microplate readings of 3 units, identical to control plate) and 22% of isolates had microplate readings of less than 6 units and greater than 3 units (fluorescence was visually difficult to detect). The remaining 745 isolates exhibited readily detectable fluorescence. Five fecal isolates that belonged to serogroup O78 were among the 22% of isolates that exhibited fluorescence that was visually difficult to detect ($p<0.001$).

Figure 1. Distribution of 455 nanometer fluoroscent readings for 1104 presumptive fecal *E. coli* isolates grown in MUG EC broth.



Curriculum Vitae

SEAN F. ALTEKRUSE, D.V.M., M.P.H.
Commander, U.S. Public Health Service, Regular Commissioned Corps
PHS Number 59953

Division of Cancer Epidemiology and Genetics
National Cancer Institute
6120 Executive Blvd, MSC 7234
Rockville, MD 20852

E-mail: saltekru@mail.nih.gov Phone: 301-594-9808 Fax: 301-402-0916

Date of Birth: Dec. 28, 1961
Location: Palo Alto, CA

Spouse: Martha H. Cox, MSW
Son: William C. Altekruse
Daughter: Leah McB. Altekruse

Board Certification

Diplomate, American College of Veterinary Preventive Medicine, 1992

Education

1998–2001	Candidate, Doctor of Philosophy (2001) Virginia Polytechnic Institute Dept. of Veterinary Medical Sciences
1987–1988	Master of Public Health University of South Carolina School of Public Health
1983–1987	Doctor of Veterinary Medicine University of Georgia College of Veterinary Medicine
1981–1982	Certificate, biological sciences University of Kent, Canterbury Faculty of Natural Sciences
1980–1983	Pre-medical Curriculum South Carolina Honors College University of South Carolina College of Mathematics and Sciences Dr. Sean F. Altekruse

1978–1980	Cambridge Advanced Level Certificate English, chemistry, biology Portora Royal School, Enniskillen, Northern Ireland

Professional Experience

Epidemiologist	July 2001–present National Cancer Institute Division of Cancer Epidemiology and Genetics Rockville, MD
	Coordinator of large vaccine trial against Human Papilloma Virus-16, the cause of most cervical cancer. The trial is being conducted in collaboration with the Costa Rican Ministry of Health.
Veterinary Medical Officer	July 2000–June 2001 Food and Drug Administration, Center for Veterinary Medicine Rockville, MD
	Conducted research on avian pathogenic *E. coli* infections in turkey flocks with an integrated turkey operation.
PHS Epidemiology Fellow	August 1998–July 1999 Epidemiology Fellowship Program Virginia Polytechnic Institute and State University Blacksburg, VA
	Training in statistics, molecular biology, and poultry medicine
Liaison	October 1994–July 1998
Host:	Centers for Disease Control and Prevention National Center for Infectious Diseases Atlanta, GA
Sponsor:	Food and Drug Administration Washington, DC
	Coordinated response, surveillance and research on foodborne infections.
Epidemiologist	March 1991–September 1994 Food and Drug Administration Center for Food Safety and Applied Nutrition Washington, DC
	Research and development of nutritional and food safety regulations.

Staff Veterinary Officer	April 1989–March 1991 U.S. Department of Agriculture, APHIS Hyattsville, MD
	Developed regulations to control Salmonella infections in eggs.
Veterinary Medical Officer	September 1988–March 1989 U.S. Department of Agriculture, APHIS Montgomery, Alabama
	Veterinary Medical Officer in the Public Veterinary Practice Career Program.
Veterinarian	July 1987–June 1988 University of South Carolina Columbia, SC
	Clinician in University Animal Care Facility.
Student extern	Spring 1987 Centers for Disease Control and Prevention
	Data collection for epidemiologic studies, viral and rickettsial zoonoses.
Student extern	Summer 1985 USDA Plum Island Research Center Greenpoint, Long Island, New York
	Technician, Insect-Vectored Diseases Laboratory.

PHS Awards

Public Health Service, Special Assignment Award, 2000
Public Health Service, Unit Commendation, 2000
Public Health Service, Commendation Medal, 2000
Public Health Service, Commendation Medal, 1999
Public Health Service, Unit Commendation, 1998
DHHS Secretary's Award: FoodNet, 1998
Public Health Service, Hazardous Duty Award, 1998
Public Health Service, Citation, 1997
Public Health Service, Unit Commendation, 1997
Public Health Service, Achievement, 1994
Public Health Service, Achievement, 1993
Public Health Service, Unit Commendation, 1992
DHHS Secretary's Award: Nutritional Labeling, 1992

Other Awards

Public Health Service Epidemiology Fellowship
Presidential Sports Award, April 2000
Ambassadorial Scholarship, University of South Carolina.

Licensure

South Carolina Board of Veterinary Examiners (1180)
Georgia Board of Veterinary Examiners (4050)

Professional Affiliations

Delta Omega Public Health Honor Society, Mu Chapter
American Association of Avian Pathologists
American Veterinary Medical Association
American Association of Public Health Veterinarians
American College of Veterinary Preventive Medicine

Public Health Service Activities

Chair, PHS Veterinary Professional Advisory Committee, 2002; Member, 2001
Guest Lecturer, Virginia-Maryland Regional College of Veterinary Medicine;
University of Georgia, College of Veterinary Medicine;
Tuskegee University, College of Veterinary Medicine;
Uniformed Services University of Health Sciences,

Publications

Journal Articles

1. Altekruse SF. Wild bird importation [letter]. J Amer Vet Med Assoc. 1991; 199:678.

2. Orosz S, Chengappa M, Oyster R, Morris P, Trock S, and Altekruse S. *Salmonella enteritidis* in two species of psittaciformes. Avian Diseases. 1992; 36:766-769.

3. Altekruse S, Tollefson L, and Bogel K. Control strategies for *Salmonella enteritidis* in five countries. Food Control 1993. Volume 4 Number 1. 10-16.

4. Altekruse S, Koehler J, Hickman-Brenner F, Tauxe R, and Ferris K. A comparison of *Salmonella enteritidis* phage types from human outbreaks and implicated egg production flocks. Epidemiol Infect 1993;110. 17-22.

5. Altekruse S, Klontz K, Hyman F, Tollefson L, and Timbo B. Bacterial foodborne infections in HIV-positive individuals. Southern Med J 1994;87:169-73.

6. Altekruse S, Hunt J, Tollefson L, and Madden J. Food and animal sources of *Campylobacter jejuni*. J Amer Vet Med Assoc 1994;204:57-61.

7. Thun MJ, Altekruse SF, Namboodiri MM, Calle EE, Heath CW, Jr. Hair dye use and risk of fatal cancers in women. J Natl Cancer Inst 1994;85:210-5.

8. Timbo B, Altekruse S, Hyman F, Klontz K, and Tollefson L. Vitamin and mineral supplementation during pregnancy. Military Medicine 1995;159:654-658.

9. Timbo B, Altekruse S, Headrick M, and Klontz K. Raw shellfish consumption in California: The 1992 California Behavioral Risk Factor Survey. Amer J Prev Med 1995;11:214-217.

10. Altekruse S, Timbo B, Headrick M, and Klontz K. Associations between diet and health behavior: results from the 1992 Rhode Island Behavioral Risk Factor Survey. J Behavioral Medicine 1995;18:225-231.

11. Altekruse S and Swerdlow D. The future of foodborne diseases. Chem Indust: London 1996;132-5, Feb 19.

12. Altekruse SF and Swerdlow DL. The changing epidemiology of foodborne diseases. Amer J Medical Sci 1996;311:23-9.

13. Altekruse S. AVMA Food safety symposium: Food and Drug Administration Response. JAVMA 1996;208:1399.

14. Altekruse S, Street D, Fein S, Levy A. Consumer knowledge of foodborne microbial hazards and food-handling practices. J Food Protect 1996;59:287-94.

15. Timbo B, Altekruse S, Mary-Glen Fowler. Breast-feeding and HIV-1 transmission: Epidemiologic studies and their limitations. Nutr Research 1996;16:759-68.

16. Timbo B, Altekruse S, Headrick M, Klontz K. Breastfeeding among black mothers: Evidence supporting the need for prenatal intervention. J Soc Pediatric Nurses 1996;1:35-40.

17. Altekruse SF. Effectiveness of consumer labels for the safety of foods of animal origin. J Am Vet Med Assoc 1996;209:2056.

18. Angulo F, Swerdlow D, Tauxe R, et al. Foodnet components. Emerg Infect Dis 1997;3:285-293.

19. Altekruse SF, Cohen ML, Swerdlow DL. Emerging foodborne diseases. Emerg Infect Dis 1997;3:285-293.

20. Centers for Disease Control and Prevention, Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters—Pacific Northwest, 1997. MMWR 1998; 47:457-62.

21. Shapiro RL, Altekruse S, Hutwagner L, Bishop R, Hammond R, Wilson S, Ray B, Thompson S, Griffin PM. The role of Gulf oysters harvested in warmer months in *Vibrio vulnificus* infections in the United States, 1988-1996. J Infect Dis. 1998;178:752-9.

22. Yang S, Leff MG, McTague D, Horvath KA, Jackson-Thompson J, Murayi T, Boeselaner GK, Melnick TA, Gildemaster MC, Ridings DL, Altekruse SF, Angulo FJ. Multistate surveillance for food-handling, preparation, and consumption behaviors associated with foodborne diseases: 1995 and 1996 BRFSS Food-safety questions. MMWR Surveillance Summaries. 1998;47—No. SS-4:33-41.

23. Headrick M, Korangy S, Bean N, Angulo F, Altekruse S, Potter M, Klontz K. The epidemiology of raw milk-associated outbreaks reported in the United States, 1973 through 1992. Am J Public Health. 1998;88:1219-21.

24. Altekruse S., Timbo B, Mobray J, Bean NH, Potter M. Cheese-associated outbreaks of human illness in the United States, 1973 to 1992: Sanitary manufacturing practices protect consumers. Journal of Food Protection. 1998;61:1405-7.

25. Altekruse S. F. *Campylobacter jejuni* in foods. J Am Vet Med Assoc 1998;213:1734-5.

26. Miller MA, Altekruse SF. The President's National Food Safety Initiative. JAVMA 1998;213:1737-9.

27. Altekruse SF, Stern NJ, Fields P, Swerdlow DL. *Campylobacter jejuni*-An emerging foodborne pathogen. Emerg Infect Dis. 1999;5:28-35.

28. Altekruse S, Yang S, Timbo B, Angulo F. A multistate survey of consumer food handling and consumption practices. Am J Prev Med. 1999;16:216-221.

29. McNeil MM, Sweat LB, Carter SL, Jr. Watson CB, Hollaway JT, Manning R, Altekruse SF, Blake PA. A Mexican restaurant-associated outbreak of *Salmonella* Enteritidis type 34 infections traced to a contaminated egg farm. Epidemiol Infect 1999;122:209-15.

30. Wallace BJ, Guzewich JJ, Cambridge M, Altekruse SF, Morse DL. Seafood-associated disease outbreaks in New York, 1980-1994. Am J Prev Med 1999:17; 48-5.

31. Trepka MJ, Archer JR, Altekruse SF, Proctor ME, Davis JP. An increase in sporadic and outbreak-associated *Salmonella* Enteritidis infections in Wisconsin: the role of eggs. J Infect Diseases 1999;180:1214-9.

32. SF Altekruse, SJ Henley, MJ Thun. Deaths from hematopoietic and other cancers in relation to permanent hair dye use in a large prospective study (United States). Cancer Causes and Control 1999;10:617-25.

33. Centers for Disease Control and Prevention. Outbreaks of *Salmonella* Enteritidis infection associated with eating raw or undercooked shell eggs—United States, 1996-1998. MMWR 2000;49:73-9.

34. Daniels NA, MacKinnon L, Bishop R, Altekruse S, Ray B, Hammond R, Thompson S, Wilson S, Bean NH, Griffin PM, Slutsker L. *Vibrio parahaemolyticus* infections in the United States, 1973-1998. J Infect Dis 2000;181:1661-6.

35. Altekruse SF, Bishop RL, Baldy LM, Thompson SG, Wilson SM, Ray BA,, Griffin PG. Vibrio gastroenteritis in the U.S. Gulf of Mexico Region: The role of raw oysters. Epidemiol Infect 2000;124:489-495.

36. Yang S; Angulo FJ; Altekruse SF. Evaluation of safe food-handling instructions on raw meat and poultry products. J Food Prot 2000;63:1321-5

37. Sobel J, Hirshfeld AB, McTigue K, Burnett CL, Altekruse S, Brenner F, Malcolm G, Mottice SL, Nichols CR, Swerdlow D. The pandemic of *Salmonella* Enteritidis phage type 4 reaches Utah: a complex investigation confirms the need for continuing rigorous control measures. Epidemiol Infect 2000;125:1-8.

Manuscripts

M.C. Evans, P. M. Adcock, T. M. Gomez, S. Altekruse, R.V. Tauxe, D.L. Swerdlow. *Salmonella* serotype Enteritidis Infections in the United States, 1985-1999: Dramatic Declines Through Successful Interventions. (Submitted to JAMA March 2001)

SF Altekruse, F Elvinger, C DebRoy, FW Pierson, JD Eifert, D Karunakaran, N Sriranganathan. Pathogenic and fecal *Escherichia coli* strains from turkeys in a commercial operation. (Submitted to Avian Diseases August 2001)

SF Altekruse, F Elvinger, FW Pierson, JD Eifert, D Karunakaran, N Sriranganathan. Antimicrobial susceptibilities of *Escherichia coli* strains from a turkey operation. (Submitted to Applied and Environmental Microbiology, October 2001)

SF Altekruse, Y Wang, F Elvinger, K Ye. Use of a statistical model to assess bacterial sample size: *Escherichia coli* strains in turkey flocks. (Submitted to Quantitative Microbiology, October 2001)

Book Chapters

Tollefson L, Altekruse S, Potter M. Therapeutic antibiotics in animal feeds and antibiotic resistance. In: N.Ahl, Ed., Contamination of Animal Products: Risks and Prevention, International Office of Epizootics, Scientific and Technical Review: 1997;16:709-15.

Altekruse S, Swerdlow D, Wells S. Factors in the emergence of foodborne diseases. In: Foodborne diseases. Vet Clin North Am (14) 1:1-15. Editor L. Tollefson. Harcourt Grace. March 1988.

Swerdlow D, Altekruse S. Food-Borne Diseases in the Global Village: What's on the Plate for the 21st Century, Emerging Infections 2. Ed: Scheld W.M., Craig W.A., Hughes J.M. 1998 ASM Press, Washington, DC. 273-94.

Slutsker L, Altekruse SF, Swerdlow DL. Foodborne Diseases. Emerging pathogens and trends. Infect Dis Clin North Am. 1998;12:199-216.

Altekruse S, Swerdlow D, Stern N. *Campylobacter jejuni*. In: Foodborne Diseases. Vet Clin North Am (14) 1: 31-9. Editor L. Tollefson. Harcourt Grace March 1988;14:31-5.Altekruse S,

Tollefson L. *Campylobacter* and related organisms. In: Foodborne Diseases. Academic Press, San Diego, CA. (In Press).

Altekruse S, Tollefson L. *Campylobacter* and related organisms. In: Foodborne Infections and Intoxications. Academic Press, San Diego, CA. (In Press).

Selected Presentations

Bayesian Analysis for identifying *E. coli* strains in Turkey flocks. Y. Wang, S. Altekruse, F. Elvinger, and K. Ye. Joint Statistical Meeting, Atlanta, GA; August 5, 2001.

A Comparison of Pathogenic and Fecal *Escherichia coli* Strains From an Integrated Turkey Operation. PHS Veterinarians Meeting, AVMA Annual Meeting. Boston, MA. July 15, 2001

A Comparison of Pathogenic and Fecal *Escherichia coli* Strains From an Integrated Turkey Operation. Northeastern Conference of Avian Diseases, College Park, MD June 21, 2001

Characterization of clinical and environmental *E. coli* isolates from an integrated turkey operation.National Antimicrobial Resistance Monitoring System, Annual Meeting, Rockville, MD March 16, 2001

Biochemical fingerprints and antimicrobial resistance patterns of *Escherichia coli* isolates from commercial turkeys. Annual Meeting of the American Veterinary Medical Association, July 23, 2000, Salt Lake City, Utah.

Salmonella serotype Enteritidis surveillance update. Northeastern Conference of Avian Diseases,

Virginia Tech, Blacksburg, VA, May 20, 1999.

Campylobacter jejuni in foods. Annual Meeting of the American Veterinary Medical Association, Baltimore, MD July 25, 1998.

Effectiveness of food safety labels for the safety of meat and poultry. Annual Meeting of the American Veterinary Medical Association, Louisville, KY. July 21, 1996.

Vibrio vulnificus illnesses in the Southeastern United States. Association of State and Federal Food and Drug Officials of the Southeastern United States. Carolina Beach, NC. April 1, 1996.

Changing epidemiology of foodborne diseases. Institute of Food Technology. Chicago, Il. November 1, 1995.

Outbreak investigations. Tuskegee University, College of Veterinary Medicine. Oct. 27, 1995.

Seafood Safety. Conference on Food Science & Technology. Auburn University. September 4, 1995

Food Allergies: An FDA Perspective. Institute of Food Technology. June 25, 1994. Atlanta, GA.

Hair dye use and the risk of fatal cancer. Results from the American Cancer Society Cohort Study. September 1993. FDA, Washington, D.C.

Strategies for the Control of Salmonella Enteritidis in five nations. 1992 Annual meeting of the Association of Military Surgeons. Nashville, TN. November 18, 1992.

Western Poultry Disease Conference. USDA-APHIS Control Program for *Salmonella* Enteritidis. March 12, 1990. Sacramento, California.