INFO SHEET

E. coli Versus Environmental Management:

Which Has Evolved Faster?

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Disease is caused by a complex interaction between the disease agent involved, the host and the environment. If the disease agent increases in number or develops strategies to evade the host's immune defenses, then disease occurs. If the environment in which the host lives, stresses or immunocompromises the host, then these disease agents are able to more easily cause disease. This infosheet will consider the host (the turkey), the disease agent (E. coli) and the environment (the turkey barn) and how these three interact to produce disease.

Colibacillosis is caused by E. coli and is a major problem for the turkey industry worldwide. The disease occurs in many forms, including colisepticemia, airsacculitis and pneumonia, together with infections of the eyes, heart, joints and bones. Avian Pathogenic E. coli (APEC) represent E. coli strains that cause invasive infections using the respiratory tract as the portal of entry. APEC colonizes the respiratory tract, penetrates the respiratory epithelium and multiplies in the bloodstream and internal organs leading to colisepticemia, the most common form of E. coli disease. The frequency and severity of this process depends on the outcome of the battle between the turkey's defenses, virulence factors possessed by the APEC and the environment which sets the stage for this contest.

Current Status of *E.coli*

Annually, a survey of veterinarians in turkey production companies within the United States (US) is conducted ranking 34 diseases of economic impact. A ranking of 1 indicates the disease or condition is not a concern. A ranking of 2 through 5 indicates the disease or condition is present with increasing severity from mild (2) through severe (5). The results of the survey are shown in table 1. Over the past 4 years, the severity and impact of colibacillosis has been consistently high. Colibacillosis increased in severity during 2006 which coincides with the ban on using enrofloxacin (Baytril®) in food producing animals within the US.

Table 1. Annual survey of US veterinarians in turkey production ranking diseases /conditions of economic impact.

	2005	2006	2007	2008
Lack of approved, efficacious drugs	4.2	4.4	4.4	4.5
Colibacillosis	3.6	4.0	4.0	3.1
Cellulitis (Gangrenous Dermatitis)	3.2	3.5	3.8	3.3
Late mortality	3.5	3.3	3.2	3.3

Table 2 shows the result of a 2006 survey of the same group of turkey veterinarians specifically focusing only on colibacillosis. E. coli is ranked as having a significant impact on mortality, morbidity, average daily gain and feed conversion. The highest incidence and severity of colibacillosis occurred in flocks 6 to 12 weeks of age with almost 20% of flocks being affected.

Table 2. 2006 Survey of US veterinarians in turkey production evaluating impact and occurrence of Colibacillosis.

Ranking	Parameter
3.5 / 3.3	*E. coli* mortality and morbidity
3.1 / 3.0	Adverse impact on Average Daily Gain and Feed Conversion
2.3	Primary Colibacillosis
2.8 / 18.4%	Impact and Frequency in turkeys 0 – 5 weeks of age
3.3 / 19.8%	Impact and Frequency in turkeys 6 - 12 weeks of age
1.8 / 10.7%	Impact and Frequency in turkeys > 12 weeks of age

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Host or Turkey Factors

The improvement in economically important production traits through genetic selection has been an ongoing and successful project. Significant gains have been made by breeding companies in breast meat yield, body weight, feed conversion and rate of gain. These faster growth rates, however, can accentuate the negative impact of disease as sick and morbid birds fall behind healthy birds within hours instead of days. As the growth rate of turkeys has increased so has the weight of the respiratory system. However, the increase in weight of the lungs is not due to an increase in the gas exchange area but an increase in the supporting structures alone. Therefore, the respiratory system of modern breeds may be more susceptible to distress and could become a limiting factor in optimal performance if damaged.

Differences in immune function have been shown between commercial turkeys of different breeds and strains within the same breed. Directly selecting birds with the most effective and efficient immune systems remains a challenge within the primary breeding industry. However, indirectly selecting birds for robustness or durability (the ability to express full genetic potential while maintaining production performance under stressful environmental conditions) is possible.

E. coli Factors

Pathogenic serotypes of E.coli have evolved some unique cellular products associated with the virulence of the organism. Most of these virulence genes are found on extra pieces of circular DNA or plasmids which reside within the E. coli bacteria. Plasmids are transferred between bacteria during conjugation (transfer of genetic material between bacteria which are touching) and typically provide a selective advantage under a given environmental state. Different types of plasmids can coexist in a single E. coli cell and may contain any or all of the following genes:

Resistance-(R)plasmids, which contain genes that can build a resistance against antibiotics (tetracycline, sulfonamides, aminoglycosides, trimethoprim) and antimicrobial agents (silver and other heavy metals, quaternary ammonium compounds. Historically known as R-factors, before the nature of plasmids was understood.

Col-plasmids, which contain genes that code for (determine the production of) bacteriocins, proteins that can kill other bacteria.

Virulence plasmids, which turn the bacterium into a pathogen (one that causes disease).

Determination of Virulence

Although there are more than 50,000 different E.coli serotypes, only a few are associated with disease. As a consequence, isolating E.coli from a clinical case is only the first step in determining the role it played in the disease process. There are a number of ways in which the virulence of an E. coli serotype can be defined such as the embryo lethality assay, polymerase chain reaction (PCR) and monoclonal antibody tests.

The embryo lethality assay is capable of discriminating among highly virulent, moderately virulent and avirulent isolates of E. coli. One of the difficulties encountered in avian colibacillosis control is that no one single identifying marker of virulent E. coli is known i.e colicin V. A large proportion of E. coli strains that produce colicin V are associated with bacteremia in humans and animals, however colicin V itself is not essential to virulence. It is the presence of plasmid Col V that carries genes that is responsible for the invasion and pathogenicity of E.coli. The Col V plasmid of the APEC strains often carries genes for drug resistance and as a result the selection pressure by antimicrobial drugs has also selected for the more pathogenic strains.

A study of E. coli from 294 clinically affected birds and E. coli from 75 apparently healthy birds showed that possession of the increased serum survival gene (iss, Table 3), was strongly associated with E. coli causing disease but not with E. coli of healthy birds. It is interesting that this gene has 87 % identity with the iss gene found in pathogenic cases of E.coli found in people. The reason for this association is not definitively known.

Some diagnostic laboratories have multiplex PCR to detect the presence of the iss, tsh, cvi and iuc genes. In addition, due to the significance of the iss gene, some laboratories have also developed monoclonal antibody testing for this gene alone.

Table 3. Summarizes the function and frequency of various genes found within plasmids of turkey APEC reported in the literature.

Genes	Function or Role	% APEC with Gene
P and F1 Fimbriae	Increased ability to bind and colonize the respiratory tract.	25 %
Thermosensitive haemagglutinin (tsh)	Agglutination of red blood cells. Colonization of respiratory epithelium.	95 %
Siderophores (SP)- Aerobactin (iuc)	Allows E.coli to sequester iron necessary for growth in iron poor environments.	74 %
Surface antigens O1, O2, O78, K1	Endotoxin production. Increased defence against phagocytes and complement that function to kill *E.coli.*	80 %
Colicin Colicin V (cvi)	Bacteriocins (proteinaceous toxins) that inhibit or kill similar or closely related bacterial strains which compete with APEC.	61 % 42 %
Increased serum survival gene (iss)	Contribute to virulence and complement resistance. Encodes resistance to ampicillin and tetracycline.	78 %
Heat-labile cytotoxin	Role unknown but theorized that they act when bird's defense mechanisms are weakened.	5.7 %
Verotoxin	Toxin causing destruction of small blood vessels, found in the digestive tract, kidneys and lungs.	11.4 %

Environmental Factors

Successful turkey producers and companies recognize that environmental management has to keep pace with the changes in genetics and nutrition in order to capture the potential of both. However due to increased production costs, many are not keeping up in some very basic areas such as biosecurity, sanitation (water and barn) and ventilation. Increased densities and decreased down -time between flocks have added to both the stress on the turkey and the number of disease organisms within the barn.

The level of *E. coli* increases in the barn environment by any of the following:

- High litter moisture and/or reused unconditioned litter
- Darkling beetles
- Ineffective water or barn sanitation
- Inadequate ventilation resulting in elevated dust and/or ammonia

In 1971, epidemiologists investigating a colibacillosis outbreak on a turkey farm showed that the serological patterns of the E. coli isolated from both the water system (a highly contaminated well, drinking troughs) and dead birds were similar. Shock chlorination of the well and consistent water sanitation, resolved the problem. Today, 38 years later, many turkey flocks still do not consistently receive sanitized bacteria free water in spite of the fact that water treatment, sanitation and monitoring systems are now readily available and our understanding of the process better.

The incidence and severity of E. coli shown in table 2 was highest in turkeys from 6 to 12 weeks of age. This is an age when birds have left the brooding barn and are frequently moved onto used litter with increased ammonia levels. Ammonia is well recognized as being able to damage the cilia in the trachea preventing it from trapping and expelling dust, bacteria and mold and also immunosuppressing turkeys. The end result is that large numbers of E .coli present within the dust are able to easily colonize and breach the respiratory epithelium then enter the bloodstream and reach the abdominal airsacs.

Disease agents and conditions which may either damage the respiratory tract allowing *E.coli* to more easily cross the respiratory lining and enter the bloodstream or which cause immunosuppression include:

- Newcastle Disease Virus
- Pneumovirus
- Avian Influenza
- Mycoplasmas
- Coronavirus
- Astrovirus
- Hemorrhagic Enteritis Virus
- Bordetella avium
- Mycotoxins from feed ingredients or poorly cleaned feedbins
- Ammonia (chronic exposure to even 10 – 25 ppm)
- Sexual maturity especially in males

These agents can all be controlled through comprehensive and effective biosecurity and sanitation programs, utilization of vaccines where appropriate and good quality feed.

If on-farm management practices are audited and found to be good yet E. coli outbreaks still occur then an analysis of the type of E.coli causing the mortality is warranted. If the APEC has several virulence genes or significant ones such as iss then specific strategies should be considered which include:

- Increased down-time between flocks
- Disinfectant susceptibility testing to ensure effectiveness against E.coli present within the environment (barn, waterlines)
- Decreasing bird density
- Use of E. coli vaccines
- Use of specific Bacillus species or bacteriophages with activity against the APEC in question

Conclusions

Not so long ago, all E. coli breaks were considered to be the result of some mismanagement or stress allowing E.coli to "take over" and cause disease. However, E. coli bacteria have been rapidly gaining an arsenal of weapons in the form of virulence genes which can cause a primary colibacillosis. Consequently, management and prevention strategies within our poultry barns to eliminate these virulent strains must evolve at a faster rate than either the genetics of the bird or the genetics of the E.coli.

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