Enteric Disease Control

Symposium Program

American Association of Avian Pathologists

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ENTERIC DISEASE CONTROL

~ SYMPOSIUM PROGRAM ~

8:00 - 8:05	T.J. Myers	Welcome.

Session I: Introduction. Moderator: Mark Dekich

8:05 - 8:15	Mark Dekich	Overview of enteric disease field problems in chickens.
8:20 - 8:30	Kenneth Opengart	Enteric diseases of turkeys - a field perspective.
8:35 - 8:50	Albert Payne	The use of computer managed databases to monitor enteric health trends in the U.S. broiler industry.
8:55 - 9:15	Edwin Moran, Jr.	Intestinal physiology influencing enteric diseases in fowl.
9:20 - 9:40	Peter Ferket	Nutritional effects on enteric disorders.
9:45 - 10:00		Break.

Session II: Immunity and Disease Mechanisms. Moderator: Robert Porter

10:00 - 10:25	Hyun Lillehoj	Role of gut-associated lymphoid tissues in local immunity to enteric pathogens in chickens.
10:30 - 11:10	Harley Moon	Pathophysiology of enteric infections.
11:15 - 11:40	Frederic Hoerr	Pathophysiology of non-infectious enteric diseases.
11:45 - 12:00		Open discussion with all Sessions I and II speakers.
12:00 - 1:30		Lunch.

Session III: Disease Diagnosis, Management and Prevention. Moderator: Hugo Medina

1:30 - 1:55	Chris Hayhow	Diagnostic approach to enteric diseases.
2:00 - 2:25	Leonard Fussell	Management and prevention of enteric diseases of chickens.
2:30 - 2:55	David Rives	Management and prevention of enteric disease in turkeys.
3:00 - 3:15		Break.

Session IV: Special Topics. Moderator: Linda Keller

3:15 - 3:40	Carita Schneitz	Competitive exclusion in poultry production.
3:45 - 4:10	M.S. McNulty	Viruses and malabsorption syndrome in chickens - some problems to be solved.
4:15 - 4:40	James Guy	Poult enteritis and mortality syndrome ("spiking mortality"): an acute transmissible disease of unknown etiology.
4:45 - 5:00		Open discussion with all Sessions III and IV speakers.
5:00 - 5:05	Y.M. Saif	Closing comments.

Message from the Symposium Organizing Committee:

This year's symposium follows and builds upon the Enteric Disease Symposium held in Orlando in 1989. That first symposium focused on the basics of both normal alimentary tract physiology as well as enteric disease mechanisms. Today's symposium will first review and update some of that material, and will then focus on how enteric diseases are controlled in today's poultry industry. We hope the information presented in this symposium will be of benefit to everyone in attendance, whether your interests are in basic science or applied medicine.

The committee would like to thank all those who made this symposium possible: the remaining members of the Enteric Disease Committee for their suggestions on topics and speakers, the AAAP Board for their financial support, Don Waldrip and Bob Eckroade for logistical assistance, past Symposium Chairs Fred Hoerr and Dennis Wages for planning advice, Kevin Fox at OmniPress for his assistance in printing the program, and, most of all, to the speakers, without whom this Symposium would not be possible.

T.J. Myers, Chair
Mark Dekich
Linda Keller
Robert Porter

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OVERVIEW OF ENTERIC DISEASE FIELD PROBLEMS IN CHICKENS

Mark A. Dekich

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SUMMARY

The essence of poultry production is turning feed stuffs into meat. The economics of the poultry industry dictates good intestinal health to achieve target growth rates and feed efficiency. Continuous, comparative performance and economic survey services (Agrimetrics, AgriTech) show the companies and poultry growing regions to be relatively close and good in these measured parameters.

Increased expertise through poultry laboratory availability and veterinary participation in day-to-day live production of poultry has improved detection, therapy, and prevention of common enteric diseases. Improved housing, nutrition, and feed quality standards has allowed poultry companies to achieve more consistent growth rates and feed efficiency.

Potential causes of enteric disease in chickens are coccidiosis, feed borne toxins, bacteria, and viruses. Subclinical problems, affecting growth rate and feed efficiency, are more common than traditional diseases that result in morbidity and mortality. Although unusual syndromes exist geographically, coccidiosis, coccidiasis/viral-bacterial subpathogens, and/or feed borne toxins are the most prevalent causes of enteric health disturbance.

INTRODUCTION

The enteric health of growing chickens is imperative to the financial stability and success of the poultry industry. The essence of the business is turning feed stuffs into meat. The purpose of the enteric system is to break down these feed stuffs into basic components for transport into the main body for growth and maintenance of the bird. Any mechanical, chemical, or biologic disturbance of this process or tissue can result in enteric disease.

GENETICS

The success of the poultry industry is based on continued genetic improvement. Base parameters of growth rate and feed conversion continue to improve. Current genetic improvements deliver a half day quicker to market (50 grams), 2 points better feed conversion, and 0.1 percent more eviscerated yield annually. The time accumulated result is bigger, cheaper, and faster growing chickens. Any disruption of the growth process is unacceptable biologically and economically. Modern chickens are hyperphagic and do not tolerate lack of feed even for short periods of time.

Geneticists working at the foundation and pedigree level are still achieving these gains on an annual basis. It takes five years for the results of their work to reach the live production level. We will see dramatic genetic improvement annually for years to come. Feed stuffs are the highest cost in production of poultry meat. Genetic improvement allows better efficiency in light of constantly rising production cost. Enteric health is paramount to maximize genetic potential.

DISEASE INCIDENCE

Chickens have few enteric diseases as compared to mammals. The incidence of enteric disease, compared to relative industry size, has decreased as general poultry husbandry, nutrition, feed quality, and veterinary surveillance improves. As the poultry industry consolidates into larger and fewer companies, more sophisticated programs for live production are employed as more technical resources are available. Although episodic enteric diseases still occur on certain farms or geographic localities, they tend to be single company limited and the result of program decisions over time.

Enteric diseases that can be easily diagnosed on gross lesions and clinical signs (i.e. coccidiosis, necrotic enteritis) are rapidly detected and treated. More subtle conditions are often "field" diagnosed or misdiagnosed (i.e. "viral enteritis") due to not employing complete and competent diagnostic testing. Such enteric problems are usually transient and tend to resolve as feed stuffs, bird populations, weather, or programs change.

Potential causes of enteric disease are coccidiosis, feed borne toxins, bacteria, or viruses. These are listed in

order of prevalence. Academically, many agents or chemicals have been tested and found to cause detrimental effects to the avian alimentary tract. This discussion will be limited to the most common agents seen on a day to day basis.

COCCIDIA

Coccidiasis is a common feature of most chicken flocks raised on built up litter in the United States. Our common chemotherapeutic control (polyether ionophores) is dependent on early leakage of oocysts to mount an immunologic response. This intracellular protozoan disrupts the intestinal epithelium and causes transient malabsorption. Whether primary or secondary, coccidiosis/coccidiasis is a common cause of enteric disease. Although veterinary monitoring for coccidiosis is intensive, recent studies question the validity of using oocyst counts, intestinal lesion scores, or intestinal oocyst levels to accurately assess disease status and predict subsequent bird performance.

With coccidiasis, gross lesions need not be present to have significant intestinal absorptive deficits. Inoculation with very low levels of oocysts ($<10^2$) will lower plasma carotenoids and lipids. Body weights are also depressed. Affected individual birds in a flock fall quickly behind. Even a small growth rate loss affects a bird's ability to compete. Weather and drug programs dramatically drive challenge levels within a geographic region. To achieve a reasonable growth rate with the current genetic potential dictates prevention of coccidiosis/coccidiasis. Current technology dictates we live with cocci.

FEED BORNE TOXINS

The second most common cause of enteric disease is feed borne toxins. Mycotoxins (trichothecene) and biogenic amines (histamine, histidine, cadaverine, putrescine) are found in feed stuffs. Excess levels of these toxins lead to field problems with enteric disease. Mycotoxins are found in grains, cereals, and soybean meal. Biogenic amines are found in rendered products such as poultry meal, meat and bone meal, and fish meal. Rancid fat can be another source of enteric disease.

Feed toxin problems are often traced back to a specific supplier. Quality assurance standards are critical in establishing minimal standards accepted for specific ingredients. Blenders of by-products are notorious for varying product quality. Consistent assaying schemes are important for monitoring ingredient quality. The most sensitive assay is biologic; the chick.

Feed borne toxins at detrimental levels result in poor performance from depressed growth rate and increased feed conversion. Clinical lesions seen may be stunting, pallor, gizzard erosion, and proventriculitis. Field problems from feed borne toxins tend to be episodic as multiple company buyers simultaneously tap the same vein of problem feed stuffs supply.

BACTERIAL ENTERITIS

Bacterial enteric disease is most dramatically seen in necrotic enteritis. *Clostridium perfringes* is ubiquitous in poultry houses. Commercial poultry are fed non-absorbed, antimicrobial products in the feed as a preventative program. On rare occasions, deteriorated animal by-products contaminated with *Clostridium sp.* may cause severe necrotic enteritis. Mostly, coccidiosis is always associated with necrotic enteritis outbreaks. Use of historic synthetic anticoccidials from the 1960's, recently interposed into conventional shuttle programs with ionophores, led to dramatic outbreaks of necrotic enteritis. The synthetic anticoccidials developed resistance quickly and severe coccidiosis breaks occurred with associated necrotic enteritis.

Increased necrotic enteritis was reported associated with use of alternative grains to corn. Wheat and barley substituted beyond 25% of corn set up the intestine for increased susceptibility to necrotic enteritis. Alternative ingredient substitution will increase as a formulation practice.

Bacterial enteritis is seen in very young flocks. Chick quality problems, overheating or an array of other stressful hatchery/management problems can induce transient non-specific bacterial enteritis. Bacterial enteritis is diagnosed secondary to coccidial or toxin problems. Laboratory confirmation of this diagnosis is often not done.

VIRAL ENTERITIS

Confirmed competent diagnosis of viral enteritis is rare in chickens. The incidence tends to be geographically confined to poultry companies in a particular area. Outbreaks may occur for short periods of time. This cause of enteric disease is often over diagnosed from the field without diagnostic confirmation. On a broad scale, viral enteritis is minor in chickens as an incidence rate.

CONCLUSION

As growth rate and feed conversion annually improve, poultry companies are more sensitive to detecting

aberrant performance numbers potentially caused by enteric problems. National composite performance figures (Agrimetrics, AgriTech) show growth rate and feed conversion improving in sync with annual genetic gains. Early detection/suppression and more sophisticated preventative programs have reduced the incidence rate of major enteric diseases. Competent diagnostics, research-based, are necessary to unravel local or geographic problems. Coccidia and feed borne toxins are the most common causes of enteric disease that affect performance today.

ENTERIC DISEASES OF TURKEYS - A FIELD PERSPECTIVE

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INTRODUCTION

As with any animal, the rate of growth and the ability of that animal to convert feed to protein mass is directly dependent on gastro-intestinal health and integrity. The turkey, however, is so efficient at both growth rate and conversion that the slightest alteration from optimal conditions is sometimes manifested as very noticeable disturbances in overall performance. What makes management of turkey enteric diseases so complex is that the impact these diseases have on the bird are easily discernable, but detecting the primary agent(s) responsible for the disturbance in growth can be challenging. Additionally, if one is lucky enough to identify a specific etiologic agent, making a true determination of the effects of these agents on growth rate in the field, and sometimes in the laboratory, becomes difficult. Obviously, prevention is the key to controlling many of these diseases. Unfortunately, we must rely on rather general control measures (cleaning, disinfection, biosecurity) in attempts to keep our flocks healthy. What is outlined below are the most widespread and economically significant enteric diseases the turkey industry contends with on a daily basis.

VIRAL ENTERITIS

Viral enteritis (poult enteritis, malabsorption-maldigestion syndrome) is probably the most frustrating, and perhaps the most common, primary cause of clinical enteric disease (28). These agents fall into the category of often incriminated, but rarely proven. Clinically, any number of enteric viruses [corona (8, 22); rota, astro, and entero (24, 25, 27); reo (27, 30); and parvolike (32), etc.] can produce an acute onset of a variety of lesions and signs including thin-walled, fluid- or gas-filled, pale, atonic intestines; diarrhea; feed refusal; dehydration; weight loss; uneasiness; litter eating; and huddling. These viruses generally affect younger turkeys, and can have what seems as an immediate but long-lasting impact on flock uniformity and overall bird resilience. What makes these viruses even more frustrating to work with is their detection, in some cases, from birds that are both clinically sick and healthy, the lack of detection from clinically ill birds, the lack of diagnostic tests available, and, as already mentioned, the inability to consistently reproduce the disease in the laboratory with purified viral cultures. Attempts at protection of poults with either transfer of passive immunity or active immunization against these agents has been generally unsuccessful. The basis of therapy is providing supportive care to affected flocks through management techniques. Control measures have, for the most part, focused on prevention of introduction of the agents to a flock (biosecurity) and cleaning and disinfecting houses following outbreaks (13, 21, 23).

The importance of viral agent(s) in what has come to be known as Poult Enteritis Syndrome (PES), previously known as Spiking Mortality, can also not be overlooked. Clinically, it appears that the early stages of this syndrome are identical to "normal" poult enteritis. For an unknown reason, a second component of this disease is a high rate of mortality, $\geq$9% from 7-28 days of age, with at least 3 consecutive days of $\geq$ 1% mortality (3). Coronaviruses and rotaviruses (especially type D rotaviruses), have most often been recovered from poults experiencing PES, although attempts at reproduction of the disease with these isolates have been somewhat inconsistent. Current attempts at control of the disease involve strict biosecurity, thorough cleaning and disinfection (including fumigation with formaldehyde), attempts at brooding on wire-floored cages, and lowering potential challenge and exposure by minimizing bird density and the number of ages of turkeys on farms.

Hemorrhagic Enteritis (HE), an adenoviral infection, causes a more unique disease in young turkeys than the other enteric viruses mentioned above. Young poults are protected from early infection by passive transfer of maternal antibody and the fact that most breeder hens are exposed naturally or to vaccine virus (6). Therefore, poults do not become susceptible to infection until at least 3-4 weeks of age, unlike other enteric viruses. Additionally, a very efficacious vaccine (autogenous splenic or tissue culture) is available that provides life-long protection against field challenge (5, 7). The vaccine was initially developed to provide protection

against field virus infection which is characterized by enlarged, marbled spleens (the primary site of viral replication); congested, hemorrhagic intestines; depression; anorexia; dehydration; and death (20). Today, however, vaccination for HE is considered as important for control of immunosuppression (15, 16, 17) leading to secondary colibacillosis, another characteristic of HE infection, as it is for protection against clinical disease. Recently, field outbreaks of clinical HE have been reported in vaccinated flocks, leading to the suspicion that there is enough antigenic variation in the field strains that the vaccine strains no longer provide adequate protection or the field virus has mutated and become more pathogenic and is "breaking through" vaccinal protection.

PARASITIC ENTERITIS

Helminths - The most significant non-protozoal parasitic infection in turkeys is by the roundworm, *Ascaridia dissimilis*. This infection has several aspects that make it important to turkey production. First, there are currently no licensed, efficacious products available for use against roundworms. Piperazine, still licensed, is only effective at at least 2 times, if not more, the labeled dosage (18). One report (34) noted an improvement in farm performance by instituting an intense anthelmintic schedule - piperazine in the drinking water for 8 hours on two consecutive days once a week during the growout period. At cleanout, after removal of the litter, sodium chloride was applied to the floors at the rate of 600 pounds per house. Another limitation of piperazine is that it only has activity against adult worms. In severe infections, adult populations may reach levels within the lumen that cause obstructional maldigestion (26). However, the larvae that are maturing within the intestinal mucosa impact intestinal health and integrity to a much larger degree (1). With no licensed anthelminthic that has larvacidal activity, and a licensed product that has lost its efficacy, controlling helminth infections is again a challenge. There has been some limited use of levamisol at 5-10 mg/lb on problem farms with good success. Additionally, fenbendazole has been reported to be an efficacious anthelmintic in turkeys when used at 18 ppm for 7 days. Both of these compounds do have larvacidal activity (19).

The other aspect of *A. dissimilis* infection that is of practical significance is its association with "white-spotted livers". Initially, this problem, which is detected at the processing plant and which causes the livers to be condemned, was thought to be due to aberrant larval migration (18). Histologic surveys of these lesions, however, suggest that some of these lesions may be due to ascarid migration and others may be due to *Eubacterium tortuosum* (2). More importantly, a large number of the small spots on livers have been identified as lymphoid nodules, a normal avian response to antigenic stimuli (31). Unfortunately, because inspection of these spots is subjective and there are no written published guidelines by FSIS, all livers found with spots continue to be condemned.

Protozoa - As with broilers, coccidiosis is, by far, the most economically important protozoal disease in turkeys, if judged by nothing other than the amount of money spent on anticoccidials every year. The importance of coccidiosis as a clinical disease in turkeys has been much harder to assess than in broilers (33). Lesions induced by the most pathogenic species of turkey coccidia are much harder to identify than those infecting broilers, and thus field assessment of the disease can become very nebulous. Coccidial oocyst shedding usually peaks between 3 and 6 weeks of age in turkey flocks (4, 33), the time when poults become the most crowded in the brooder house, litter management becomes more difficult, and overall stress is at its highest. The significance of peak oocyst shedding at that time on performance, however, is yet undetermined as one study was unable to correlate any performance factor with oocyst counts (33). In fact, there are still those that argue that coccidiosis in turkeys is a minor problem. Nevertheless, anticoccidials are still used by all integrators, although some claim it is more the growth promoting effects of the compounds than their anticoccidial activity.

Histomoniasis (blackhead) can cause significant morbidity and mortality in turkeys. With the withdrawal of the registration of the nitroimidazoles, which were highly effective in the treatment of histomoniasis, the industry has been left with no effective treatment for the disease. Fortunately, the incidence of histomoniasis has remained low and the need for effective therapeutic agents has been minimal. If this were to change, the industry could have a very big problem with this disease. Prevention is generally focused on eliminating exposure of turkeys to cecal worms or earthworms, the two primary vectors for the protozoan parasite. Confined rearing has done the most to prevent this exposure. The elimination of contact between chickens and turkeys also aids in the prevention of histomoniasis as chickens can harbor large number of cecal worms.

Other significant protozoal agents include cryptosporidia (10), trichomonas and cochlosoma (12). These agents are often encountered as sequelae to viral enteritis and PES. There is debate as to whether these agents have a primary

role in the production of enteric disease, or if their role is strictly as secondary invaders. There are few, if any, therapeutic agents for these protozoal infections. Roxarsone has been used in cases of trichomonad infection with limited success. Therefore, treatments are usually supportive and prevention usually dependent on cleaning and disinfection of premises with an emphasis on biosecurity.

BACTERIAL ENTERITIS

Paratyphoid infections do play a role in some of the early enteritis commonly seen. It is not uncommon to isolate these organisms from the intestines of birds with "poult enteritis" within the first 2-3 weeks after hatch, as wells as apparently unaffected poults. Whether these organisms are the primary cause of the enteritis or the populations thrive in an unhealthy intestinal environment as a result of viral infection is hard to discern. Also unclear is whether feed, environment, or indirect vertical transmission is the primary source of these agents. There is also the more characteristic salmonella infection that has with it the characteristic cecal cores, coagulated yolks, congested viscera and, occasionally, pericarditis (11). These outbreaks are usually associated with a breeder flock source, egg shell contamination, and hatchery dissemination, and are far less common. Lukas and Bradford (11) found that 21% of paratyphoid outbreaks in California were the more severe type of infection and 62% of the infections were catarrhal enteritis where salmonella was the only agent or acting with other infectious agents to produce enteritis. Paratyphoid serotypes frequently isolated include *S. hadar*, *S. heidelberg*, *S. st-paul*, and *S. typhimurium*, although it should be noted that serotypes can be very geographically specific.

Clostridial enteritis is a more poorly defined entity in turkeys than in broilers. Classic necrotic enteritis is rarely seen, although treatment of poults experiencing enteritis with gram positive spectrum antibiotics often seems to improve their overall appearance. Whether these therapeutic agents are acting specifically on a clostridial population that has taken advantage of a poor intestinal environment or they are acting in a more general way to improve the enteric environment is, again, difficult to discern.

The role that long, segmented, filamentous organisms (LSFOs) play in enteric problems in poults is very unclear. Some studies have reported diarrhea (9) and a reduction in rate of gain (14) in turkeys infected with these organisms. Other researchers have concluded that they are not a causative agent of enteritis by themselves, but were unable to speculate on what their significance might be.

DIETARY ENTERITIS

There continue to be many discussions about the role certain feed ingredients and feed ingredient quality may play in enteritis. Sell (29) noted that soybean meal and supplemental fat may be involved in some of the intestinal disturbances of young poults, and that sunflower meal was consistently able to alleviate some of the negative effects of stunting syndrome. Other factors which are discussed as possibly inducing enteritis are rancid fat, mycotoxins, and biogenic amines. All of these have been a challenge to characterize in finished feeds and, therefore, it has been difficult to link them with specific cases of enteritis.

MISCELLANEOUS ENTERIC CONDITIONS

Flushing, characterized by the passing of excessively wet droppings, is, perhaps, one of the most misunderstood, but most prominent enteric conditions of turkeys today. Causes can include infectious agents, dietary factors, environmental management, or toxins. Severe episodes of flushing in older turkeys has been associated with younger birds on the same farm being affected by PES (3). In severe cases, older birds will, in as short as a 24 hour period, consume massive quantities of water and then totally saturate the litter within the house.

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THE USE OF COMPUTER MANAGED DATABASES TO MONITOR ENTERIC HEALTH TRENDS IN THE U.S. BROILER INDUSTRY

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INTRODUCTION

It is common for the U.S. broiler industry to perform routine health monitoring examinations. These examinations, routinely referred to as posting sessions, serve as one of several tools used in preventative medicine. Historically, this information has been collected on a routine basis for the purpose of evaluating anticoccidial efficacy and flock health. As with the monitoring of other aspects of the broiler industry, the continuous accumulation of large amounts of raw data has hindered attempts to objectively evaluate and interpret health trends. Posting information is often summarized subjectively at the end of each session and may never be used again.

In recent years, spreadsheets have been designed to evaluate poultry health data. Although spreadsheets can tabulate data, they lack both the utility to manage large volumes of data and the flexibility to make relational comparisons across categories and over time. The inability to perform these functions makes the evaluation of disease interactions, seasonal influences, and bird health programs very difficult.

By using a relational database system that manages large volumes of categorical data, disease trend analysis can be achieved. Several years ago Elanco Animal Health developed such a system to allow for better use of data obtained during broiler posting sessions. With this system, most available computer hardware can now be used to provide timely customized reporting of health, production, and management data. The ability to analyze and interpret the enormous amount of data collected during regular posting sessions will result in a better understanding of the trends and opportunities in the area of broiler health and performance management. The Necropsy Tracking System (NTS©) developed by Elanco contains information collected from more than 15,000 birds annually. This information, collected and evaluated by Elanco's technical consulting staff, provides health trend comparisons over time, and within and between geographic regions.

METHODS

In this paper we will focus on intestinal health data collected over the past eighteen months. This enteric evaluation includes conditions such as coccidiosis, enteritis, gizzard erosion, proventriculitis, and oral lesions. All data are collected in a numerical format. In a typical posting session, birds are brought into a central location for post mortem evaluation. Typically, five to ten birds ranging in age from fourteen days to processing age are evaluated in each house. In a one million bird complex, the posting will often represent approximately one hundred birds from ten to twenty farms. Coccidiosis scoring is based on the widely used method described by Johnson and Reid[1]. Enteritis, gizzard erosion, and proventriculitis are based on a score of 0 to 3 (0 = normal or none present, 1 = mild, 2 = moderate, and 3 = severe). Oral lesions, suggestive of mycotoxin exposure, are scored as present or not present and are reported as a percentage of birds with lesions. Coccidiosis, enteritis, gizzard erosion, and proventriculitis lesions can be reported as either percentage of incidence or mean score.

RESULTS

A summary of the current NTS© database reveals that on a national level the average age of birds posted was thirty days. Of the three major species of coccidia seen in broilers, *E. acervulina* is the most common with approximately twenty-three percent of the birds exhibiting lesions, depending on the season of the year. The incidences of *E. maxima* and *E. tenella* were ten and one percent, respectively. Enteritis was seen in twenty-seven percent of the birds and the incidence of proventiculitis and oral lesions were each ten percent. Gizzard erosion had a much higher incidence with forty-five percent of the birds exhibiting lesions. Although regional differences were observed, these differences

were not as significant as previously thought. When evaluating lesion severity, gizzard erosion and enteritis appear to be the major enteric diseases. The most consistent influence on enteric disease is seasonal, with coccidiosis being greatest in spring/summer and enteritis and gizzard erosion being most severe in fall/winter. There is also a marked difference due to the bird age. Gizzard erosion, enteritis, proventiculitis, and mouth lesions all increase with age. However, *E. acervulina* shows a quadratic effect that peaks around four weeks of age and virtually disappears by forty days of age. While the incidence of mouth lesions gradually increases with age, a dramatic increase is seen around four weeks of age. These results provide a better understanding of the normal disease incidence in the field and serve as the basis for specific investigations into enteric health.

CONCLUSION

When evaluating data collected during posting sessions, a clear understanding of industry trends can provide better recognition and insight into specific problems related to enteric health and flock performance. Consideration should be given to the age of bird evaluated, the pattern of the disease process, the season of the year, and how the incidence of the disease compares with normal occurrence in that region. Posting information containing a large volume of interactive data is best evaluated and interpreted using a relational database. Using Elanco's NTS© one can not only evaluate the incidence and severity of a specific disease, but can observe changes over time and region and can identify relationships between health parameters. Management of the data is critical in maximizing the value of time and resources spent on monitoring broiler health.

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INTESTINAL PHYSIOLOGY INFLUENCING ENTERIC DISEASES IN FOWL

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SUMMARY

Mucosa and motility are central to intestinal physiology. Lumenal exposure in the small intestine is extensive because of dense elongated villi and enterocytes with microvilli. Mucin from goblet cells creates an unstirred water layer in the microvilli glycocalyx. This layer is a barrier to particulates and microflora while allowing products of pancreatic digestion in the lumen access to surface enzymes that yield absorbable molecules. Muscle fibers in the mucosa facilitate villi movement while extensive refluxive peristalsis by the circular muscle layer also facilitates convective exchange.

The large intestine presents minimal surface area and motility is more oriented to the separation of fluid and fines from coarse particulates than surface convection. Essentially, indigesta in the colon (after ileal transfer) is manipulated by retroperistalsis carrying urine from the cloaca. Small orifices restrict entry into the ceca to fluid and fines. The proximal ceca have extensive villi where active mechanisms can recover electrolytes and water; whereas, the distal ends have a large lumen, minimal surface, and gentle peristalsis to enable an anaerobic population to generate volatile fatty acids from labile fiber.

Mucosa in place with the embryo undergoes transition during the last third of incubation to a mature surface 2-3 weeks after emergence. Small intestinal enterocytes with the embryo do not engage in nutrient retrieval as much as immunoglobulin transfer and expansion of the vascular system. Enterocytes favoring digestion-absorption subsequently arise from the crypts to create a mosaic with embryonic forms until extrusion from villi tips. Concurrently, embryonic enterocytes in the large intestine are fully competent at active transport, and the bulk of this activity is lost with replacement by adult forms and development of a microbial population.

The intestine evolved under a considerably less intensive environment than commercially exists. Variabilities in feedstuffs and microbial load create inordinate opportunities for enteric threats, particularly during the embryonic to adult transition.

INTRODUCTION

Nutrient recovery from digesta in the lumen represents the primary objective of the intestine. Mucosal characteristics and coordinated motility are central to this objective. The small and large intestine differ markedly with respect to nature of mucosa, type of motility, and nutrients absorbed. The following is a cursory description of intestinal physiology in mature fowl, and its transition from the embryonic state with hatching. Pertinent and recent references are given to provide the reader additional information.

SMALL INTESTINE

Enterocytes provide nearly all of the lumenal exposure. Their structure and histochemistry in fowl are a direct parallel to all other animals (22). Goblet cells are minor in number by comparison, but they are important to overall intestine operation. Pastor *et al.* (30) reported two types of mucus producing cells. Most abundant are the ones producing sialo-mucins; whereas, a second type contains both sialo- and sulpho-mucins, and increases in number from duodenum to the ileo-cecal juncture.

Microvilli greatly expand each enterocyte's surface exposure. Contractile elements provide convective movement (23), whereby immobilized enzymes (20) finalize digestion and improve the likelihood of absorption. In association with the microvilli are also an array of N- and O-linked glycoproteins that act as lectins and are speculated to have significance in parasite interactions (1).

Mucin released from nearby goblet cells is entrapped within the glycocalyx-microvilli network to create an "unstirred water layer" at the surface (27). This viscoelastic gel acts as a three dimensional screen which excludes all particulates and restricts diffusion of large molecular weight compounds (14). Such a "screen" serves two purposes. Solubilized nutrients can approach the absorptive surface where finalization of their

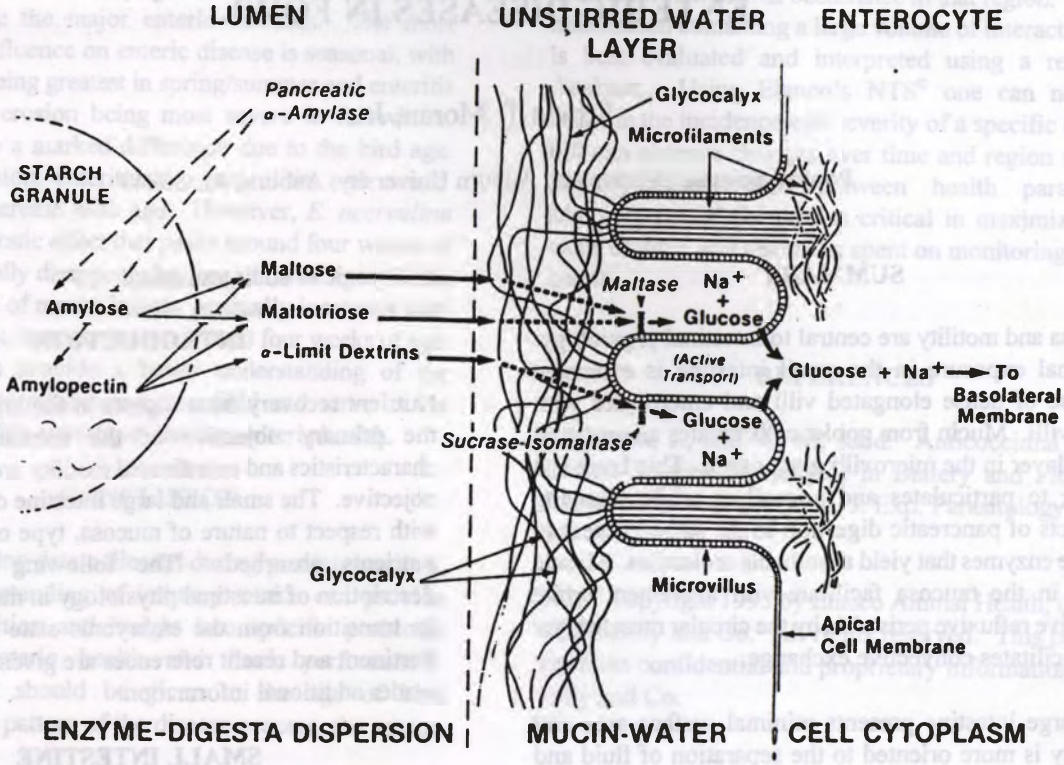

Figure 1. Postulated sequence of events in the digestion of starch by fowl. Starch is normally in granular form, and pancreatic α-amylase progressively hydrolyses constituent amylose and amylopectin to maltose, maltotriose and α-limit dextrins. Enterocytes lining the small intestine project microvilli and a fibrous glycocalyx into the lumen. An aqueous dispersion of mucin from nearby goblet cells is immobilized in these structures to form the "unstirred water layer." Dissolved products from starch digestion must diffuse through this barrier to reach carbohydrases anchored on the surface in order to finalize digestion; however, glucose accrues near active transport sites, and its rate of absorption is improved. Exclusion of glucose from the lumen also limits access to associated microflora and their growth. (From Moran, 1985).

digestion creates focal concentrations of product that do not dissipate. Associated enzymes are protected from degradation by pancreatic enzymes in the lumen (Figure 1). Transient microbes in the lumen also do not have ready access to these particularly labile products.

Villi adaptation to diet depends on cell turnover from proliferative activity in the crypt. Enterocytes differentiate structurally to become competent at digestion-absorption as they ascend the villus (12). Competence does not occur until mid-point (19); then extrusion from the tip eventually occurs, whereupon their enzyme array contributes a small amount of digestive activity to the lumen (17). Enterocyte enzyme complement can adapt to meet dietary needs (29). Similarly, villus length can be altered to optimize nutrient recovery with dietary dilution, microbial competition, and loss of enterocyte capacity because of parasitization (8,33,39).

Motility facilitates nutrient absorption by maintaining a maximum concentration differential between lumenal contents and the unstirred water layer. A well developed nervous system (2) coordinates bolus progression to balance energy expenditure with nutrient recovery. Fowl employ a to and fro refluxing as a means of convection which lends to a prominent circular muscle (42). Such refluxing is particularly active between the gizzard and distal duodenum where bile and pancreatic juice enter (7,28,38). High environmental temperature reduces the extent of motility and rate of nutrient uptake, as do circumstances that increase viscosity of lumenal contents (43).

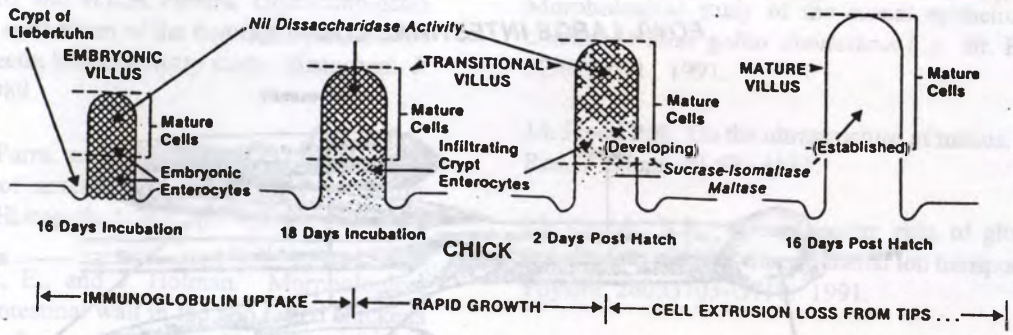

Figure 2. Cellular changes postulated to occur on the villus during perinatal development of the chick. Enterocytes placed on the villus during embryonic development are oriented to immunoglobulin transfer. Immunoglobulin uptake stimulates villus growth which, in turn, depends on enterocytes formed in the crypt. Maturation of crypt enterocytes leads to the appearance of carbohydrases capable of digesting plant saccharides. Competence to utilize starch progressively develops with dominance of crypt enterocytes on the villus and eventual displacement of those from embryonic origin.

The embryo's small intestine is incapable of digestion and active absorption at its surface. Transition to competence is initiated during the last third of incubation and is complete 2-3 weeks after hatching. Although embryonic enterocytes histologically resemble adult cells (16), digestive enzymes are absent from the surface, as are mechanisms for active transport. Competence in both respects arises from "new" cells generated in the crypt (Figure 2). As a result, villi grow markedly during the first week after hatch (3); in turn, small intestinal growth dominates total GI tract development (9). Yamauchi and Isshiki (45) observed that villi shape change from finger-like at one day of age, to wave- or tongue-like 30 days later. Bayer *et al* (4) noted discontinuities in the mucosa early in development, and goblet cells became less apparent as the chick aged.

LARGE INTESTINE

Objective of the large intestine is to salvage nutrients remaining with indigesta from the small intestine together with those excreted in the urine. Essentially, a coordinated effort of motility, microflora, and mucosa recovers water, sodium, and volatile fatty acids.

Motility coordination is central to function. Indigesta from the ileum is ejected into the colon through the ileo-colonic sphincter (Figure 3). Sphincter opens and closes rapidly to maintain a low microbial level in the small intestine where operations favor aerobic conditions, as compared to the large intestine where a dense population depends on an anaerobic environment. Gentle retroperistalsis starting in the urodeum transfers urine and fine particulates through small orifices into the ceca (5,11). Coarse particulates segregate to the colon core and progress to the coprodeum where a critical mass accrues for defecation.

Microflora associated with the large intestine are diverse strict and facultative anaerobes that vary in population representation to accommodate the nature of indigesta (34,35). Fermentation favors the neutral detergent type fiber and formation of volatile fatty acids which, in turn, improves recovery of associated water and electrolytes (21,36).

Ceca provide the greatest lumen volume for fermentation and absorptive surface. Cecectomy adversely influences the water and energy economy of birds (6). The proximal ends of ceca have mucosa with well developed villi and low luminal volume; whereas, distal ends have greater luminal volume but minimal villi (13). Enterocytes at the proximal end have distinct microvilli and goblet cells with the converse situation occurring distally (10).

Absorption of water and electrolytes is influenced by the extent of dehydration and need, particularly for sodium (40). Aldosterone has been shown to be a major factor in mucosal activity (37). Volatile fatty acid uptake may be either passive or active with involvement of sodium throughout the ceca (15). Active transport of glucose declines from proximal to distal ends as the nature of

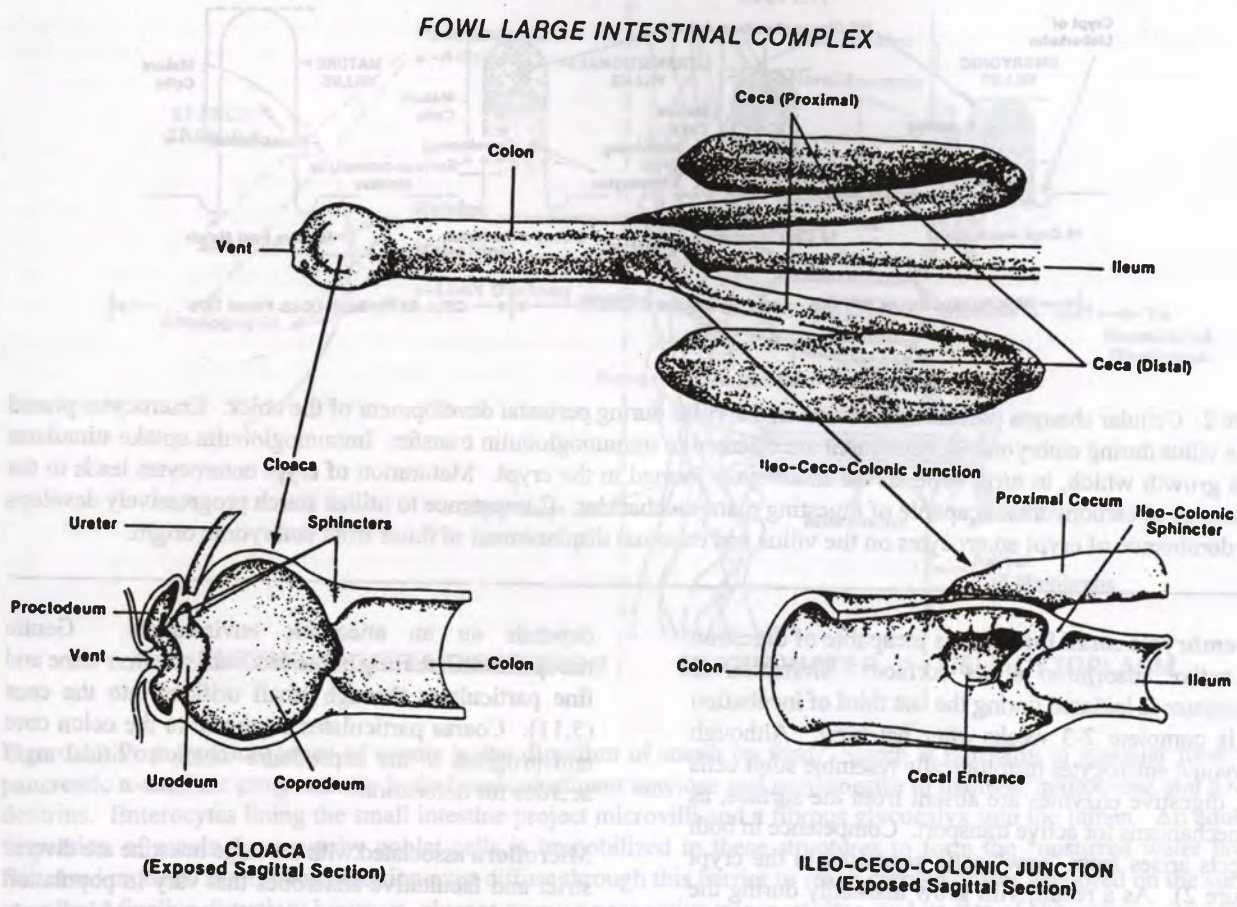

Figure 3. The fowl's large intestinal complex is comprised of colon, ceca, and cloaca. Essentially coordinated motility moves fluids (from urine in the cloaca, together with fine particulates from indigesta propelled into the colon) through small orifices that connect ceca. Microbial fermentation generates volatile fatty acids which are absorbed by mucosa, together with water and electrolytes. (From Moran, 1982).

surface changes (32); however, practical significance seems moot given concurrent microbial competition for these labile nutrients.

Embryonically formed cells throughout the ceca are fully functional at active transport of nutrients (26). As lumen microflora accrue after hatching (18), and fermentation becomes active (44), absorptive cells in the distal region loose active transport processes (31). Presumably, the large intestine acts after hatching as a "backup" in nutrient recovery when the small intestine is lacking.

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NUTRITIONAL EFFECTS ON ENTERIC DISORDERS

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INTRODUCTION

Enteric health and nutrition are intimately related. Poor enteric health can adversely affect food digestion, gut motility, and nutrient absorption by several means. Likewise, poor nutrition and feed quality can either increase a bird's susceptibility to enteric disorders or directly cause them. Often it is difficult to discern the true cause of enteric disorders in poultry, whether it be pathogenic or nutritional in origin. My objective is to present general information on common nutritional factors that influence gut health, including feed intake, palatability, feed ingredient quality, and feed formulation. Since enteric disorders are possible with even the best nutrition program, I will conclude with some practical intervention strategies to minimize the adverse effects of enteric problems.

FEED INTAKE

Feed intake is one of the first things a grower will see change when poultry succumb to some kind of enteric disorder. Like all animals, poultry will not eat normally when they do not feel well. People often confuse this behavior with feed refusal associated with a feed quality problem. To discern whether feed refusal is due to poor feed quality or infectious disease, one must have a broad perspective on the situation. If the feed refusal problem is company-wide, then feed quality is usually the suspected cause. If the problem is only among a few farms, then disease management is usually the suspected cause. Do not be quick to judgment until all the facts have been carefully studied.

Maintaining feed intake is especially important to avoid mortality or severely stunted growth when birds are afflicted with an enteric disorder. In Poult Enteritis and Mortality Syndrome (PEMS), for example, one of the very first symptoms observed in a flock about 24 hours before the diarrhea starts is a distressful vocalization and impatient pacing behavior along the feeder lines. The poults seem to be disturbed about the feed, and they will not eat it. The spike in mortality usually does not occur until about 3 days later when many of the birds begin to consume feed again. A similar response can be observed in broilers subjected to a sudden decrease in the duration of light exposure after 14 days of age. After about 14 days of age, the diurnal feeding behavior in broilers is established and a change in lighting program will adversely affect feed intake in some birds. For example, if a bird is accustomed to feeding during a time that has changed from a light to a dark period, it will go hungry for several days until it learns to eat during the light period. Often, a spike in mortality follows shortly after the birds begin eating, and dead birds are often found with their intestines full of feed. In other words, realimentation following a period of inanition due to management or enteric disorder can cause a spike in flock mortality.

This spike in mortality is associated with hypophosphatemia (Frank Edens, 1996, personal communication, North Carolina State University, Raleigh, NC). While the birds are off feed, their phosphate reserves deplete. Thus, when they begin to consume feed again, the high demand to phosphorylate the influx of glucose for ATP production in the liver and muscle tissue drains blood phosphate reserves to fatally low levels. This response is especially evident during stressful conditions, which require a cascade of ATP phosphorylation. In addition, it is common for animals to develop respiratory alkalosis and metabolic acidosis during periods of starvation, and this will also cause a shifting of phosphorus into cells (Mostellar et al., 1964). This phenomenon is thought to be created by the phosphate trap resulting from glucose-6-phosphatase deficiency leading to type I glycogen storage disorder. Therefore, it is important that a grower take every measure possible to keep the afflicted poults eating to maintain blood phosphorus and other critical nutrients. In realimentation after starvation, hypophosphatemia can be induced by inadequate dietary phosphorus. Therefore, top dressing the feed with a highly available source of phosphate or supplementation of drinking water with a soluble source of phosphorus when the poults show the first signs of feed refusal may help prevent the birds from becoming hypophosphatemic and dying upon realimentation.

PALATABILITY

Palatability of the feed is extremely important to maintain feed intake of young birds (especially poults) when they experience an intestinal upset. Palatability is more influenced by textural properties than by flavor because poultry have few taste buds and a relatively dry mouth, especially when they are dehydrated because of diarrhea. Small firm pellets and good crumbles with few fines is ideal to maintain palatability. Too much dry fines in feed is difficult for birds to consume, much like it is difficult for us to eat a big mouth full of dry crackers without something to wash it down. Dumping feed pans, cycling feed lines, and getting the birds up and moving frequently may encourage feeding activity. Some people claim that afflicted birds can be encouraged to eat by top dressing the feed with grit, coarse ground corn, whole kernel wheat, whole rolled oats, powdered milk, molasses or confectionery sprinkles. Starter diets containing coarsely ground corn is better than finely ground corn because it results in less fines in the crumbled feed and the poults are able to freely select the high carbohydrate ingredient if they need it. Diets containing high fat (about 7.5% total dietary crude fat) improve feed palatability by reducing fines and increasing oral lubrication. It may also slow food passage rate, thus improving feed utilization.

PROTEIN AND AMINO ACIDS

Dietary crude protein level and amino acid balance can influence the susceptibility of poultry to enteric disorders by affecting nitrogen metabolism and enteric microflora. Excess crude protein or poor amino acid balance can be a metabolic stress to chicks and poults, thereby adversely affecting feed intake, feed conversion, and growth. Excess dietary protein and amino acid forces the birds to eliminate nitrogen *via* the kidneys, which elicits a flushing (diuresis) response. Excess dietary protein may also shift the microflora to a more proteolytic population (i.e. clostridia), which can adversely affect gut health. Amino acid balance can be improved by reducing dietary crude protein to about 90% of NRC recommendations and supplementing with lysine, methionine and threonine.

DIGESTIBILITY

Digestibility of feed ingredients may influence the severity of enteric disorders in poultry. In general, poorly digested feed results in less nutrients being available to the bird for growth and more nutrients available for gut microflora and pathogens; this is not good. Poor feed digestibility can be due to several antinutritional factors in feed ingredients: 1) enzyme inhibitors (i.e. trypsin inhibitor in underprocessed beans); 2) nonstarch polysaccharides in grains; 3) overcooked protein; and 4) poorly digested protein sources (i.e. keratin in feather meal or hair). For example in turkeys, a significant portion of the protein in starter diets comes from soybean meal. Too much dietary soybean meal (>30% of the diet) may cause malabsorption or maldigestion because of the high dietary level of the osmotically active non-starch oligosaccharides, galactomannans, raffinose and stachyose. Similar problems exist in feeding high amounts of grains containing non-starch polysaccharides such as β-glucans, arabinoxylans, and other pentosans. Poultry, especially young ones, do not have the innate enzyme capability to digest these carbohydrates, which ultimately increases microbial fermentation. Moreover, these compounds alter gut digesta viscosity, which adversely affects digestion and nutrient absorption, or they indirectly irritate the gut by increasing microbial fermentation. Dietary supplementation of enzymes, such as α-galactosidase or an endomaninnase for soybean meal, β-glucanase for barley, and arabinoxylanase for wheat may alleviate the adverse effects of excess non-starch polysaccharides on birds subject to enteric disease.

FIBER, FAT, AND TEXTURE

Dietary fiber, fat, and feed texture all influence the severity of enteric disorders by modifying gut motility. Feed passage rate generally increases as dietary fiber content increases, and passage rate decreases as dietary fat content increases. However, in young poultry with an enteric problem, these dietary components may help normalize gut motility. Good gut motility is necessary for proper food digestion, nutrient absorption, and maintaining a healthy gut environment. Dietary fiber and feed texture (particle size and integrity) are important for proper gizzard motility. The gizzard is the "pace-maker" of normal gut motility (Duke, 1994). Unlike mammals, vigorous gut refluxes (i.e. backward flow or reverse peristalsis) are normal in birds as an adaptation to compensate for a short intestine. The refluxes serve to re-expose intestinal digesta to gastric secretions, vigorously mix digesta with enzymes to enhance digestion, and discourage microbial proliferation that may cause disease or compete for nutrients. Dietary fat stimulates the reflux of digesta from the jejunum through duodenum into the gizzard, thus slowing food passage rate and improving the utilization of dietary protein and energy.

TOXIC COMPOUNDS

A diet free of toxic compounds should be the standard objective for every feed manufacturer. A strict quality control program will reduce the risk of inadvertent

contamination of feeds with mycotoxins, phytotoxins, biogenic amines, pharmaceuticals, and other toxic compounds. Mycotoxins may cause enteric disorders, feed refusal, and malabsorption. Oxidized fat has deleterious effects on the gut health of poults (Dibner et al., 1995); so, poultry should be fed the highest quality fat possible and a liberal amount of antioxidant to prevent the development of fat rancidity.

The biogenic amines, histamine and tyramine, can cause or aggravate an enteritis problem if they exceed about 100 ppm in the diet. These amines accumulate in spoiling meat and fish by-products. Fish meal is a common source of biogenic amines; but, excessive levels of biogenic amines rarely occur if the dietary inclusion rate of fish meal is below 5%. Since there is no rapid or inexpensive test for biogenic amines, it is necessary to purchase high quality fish or animal by-product meals. Be suspicious of protein by-product meals that are much darker in color than normal, or which have putrid, sweet, pungent, or ammonia odors. Low quality protein meals (e.g. over-cooked meals) are poorly digested in the foregut, and thus pass into the hindgut where they are degraded by proteolytic bacteria (e.g. clostridia, *E. coli*, *etc.*). These proteolytic bacteria produce biogenic amines and other toxic compounds. If possible, feed the highest quality protein by-product meal in the starter diets.

INTERVENTION STRATEGIES

Strict biosecurity practices are extremely important in the prevention of enteric disorders. However, in concentrated areas of poultry production, even the best biosecurity practices may not prevent enteric problems. Usually, affected birds will show a peculiar disturbed behavior, and diarrhea starts the next day. Any abnormal behavior is the first clue that the birds may have an enteric upset and intervention treatments should be started immediately to reduce subsequent diarrhea, dehydration, and eventual mortality. The following are some suggestions for managing birds through an episode of enteritis or diarrhea. These suggestions are particularly helpful in helping turkey poults through a case of Poult Enteritis and Mortality Syndrome (PEMS).

1. Give the birds milk replacer *via* the drinkers. When the birds start "running and hollering", turn the water off for 2 - 3 hours to build a thirst. Then mix calf milk replacer according to the directions and dispense it into the drinkers until all the birds have had a chance to consume their fill. Then, return the birds to *ad libitum* consumption of water (optionally) supplemented with electrolytes or a rehydration solution. Repeat this treatment for three consecutive days. The milk replacer, which contains casein that can coagulate in the gut, "tightens" up the birds (controls diarrhea). The milk replacer also supplies sufficient lactose to encourage the proliferation of lactobacilli and other favorable bacteria which act to competitively exclude pathogenic organisms. Moreover, the milk provides a highly available source of phosphorus and other important nutrients for the bird. Be careful not to give the birds too much milk replacer because supplying too much lactose will cause osmotic diarrhea. Do not substitute powdered milk, as used during water vaccinations, for the calf milk replacer. Powdered milk does not contain sufficient casein and other important nutrients, so it is not as effective in controlling the diarrhea.

2. Give the birds a rehydration solution. Birds lose a lot of electrolytes during diarrhea, particularly sodium, potassium, and chloride. If serious diarrhea persists, the birds should be given the rehydration solution to replenish these important electrolytes or at least slow their depletion rate. The World Health Organization (WHO) formulation, which contains electrolytes and glucose, is a good rehydration solution. The rehydration treatment should be done every 6 hours until the diarrhea stops. The rehydration solution formulation is shown on Table 1. If the birds are not better after consuming the recommended dosage, then repeat the dosage without interruption.

3. Supplement drinking water with a commercial electrolyte pack if the diarrhea is not very serious. This treatment helps prevent excessive loss of electrolytes

Table 1. The World Health Organization rehydration solution formulation for the treatment of diarrhea.

WATER	128 GALLONS	1000 LITERS
SALT (NaCl)	3.7 POUNDS	3.5 KG
SODIUM BICARBONATE	2.7 POUNDS	2.5 KG
POTASSIUM CHLORIDE	1.6 POUNDS	1.5 KG
DEXTROSE	21.3 POUNDS	20 KG

during mild diarrhea. Sometimes a mild diarrhea causes a perturbation in electrolyte balance, which in turn causes a chronic, more severe case of diarrhea. Vitamin supplementation packs containing the B-complex vitamins and vitamins A, D, and, E can help the poults endure the stress of enteritis. Also make sure the amount of vitamin A does not exceed 8 times the level of vitamin D. Excess vitamin A will compete with the absorption of vitamins D and E, resulting in rickets or immune deficiency problems.

4. Use antibiotics according to the recommended program. Treatment of the flock with Sarafloxacin Hydrochloride (SaraFlox®WSP, Abbott Laboratories, Inc., North Chicago, IL) has been shown to be effective in stopping the mortality, but it may not prevent the growth depression associated with PEMS and similar enteric disorders. Treatment for the recommended full 5-day period is very important to preventing pathogen resistance to this medication. I recommend that the birds be given a probiotic-type product immediately after a therapeutic antibiotic treatment to stabilize the gut microflora.

5. Use whole wheat, crimped oats, or coarse ground corn in starter feed. Often birds exhibit feather and litter picking during an enteric upset. I think this behavior is the bird's attempt to "jump-start" or stabilize gut motility, which is greatly influenced by gizzard function. Young birds can consume whole wheat, which is much more nutritious than feathers or litter, and it should do a better job of satiating the gizzard. I recommend the use of about 5% wheat or clean oats added to the feed on the farm by dumping 50 to 100 lb. of wheat into the feed line hopper. Some turkey companies have also found that coarsely ground or cracked corn in the starter diet, or whole corn in growing and finishing diets, is helpful to encourage feed consumption and stabilize gut motility. The birds should be observed to see if they are attracted to whole grains. If they show an interest in the whole grains, it will probably be helpful. This treatment will also help birds that show some degree of feed refusal. Free-choice grit along with the whole wheat or cracked corn may also be helpful, but perhaps not necessary.

6. Betaine supplementation of the starter diets or drinking water may help prevent or treat some of the adverse effects of enteric disorders. Betaine is an important metabolic factor involved in the absorption and utilization of fat, it is necessary for rapidly growing tissue (especially the absorptive surface of the gut and feathers), and it is involved in osmoregulation (Garlich, 1995). All of these functions are adversely affected by enteritis. Ferket (1995) reported that betaine may help control or prevent diarrhea in poultry by modulating osmoregulation. Dietary supplementation of betaine should be done at a rate of 1 to 2 kg betaine/ton in starter feed or in feed fed when diarrhea (flushing) occurs in older birds. Drinking water supplementation of betaine to treat diarrhea should be done at a rate of 500 mg/kg body weight over a 2- to 3-day period for maximum efficacy.

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ROLE OF GUT-ASSOCIATED LYMPHOID TISSUES IN LOCAL IMMUNITY TO ENTERIC PATHOGENS IN CHICKENS

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SUMMARY

The intestine constitutes a large proportion of the animal's immune system. Both resident and migratory lymphocytes located in the intraepithelium and lamina propria play an important role as the first line of defense on mucosal surfaces. Expression of mucosal immunity is mediated by various T lymphocyte subpopulations through production of soluble cytokines and lymphokines. Furthermore, various selectins and integrin adhesion molecules mediate lymphocyte migration to the gut. Advances in our understanding of the development and function of the intestinal immune system will soon enable the development of novel vaccination and immunization strategies for control of intestinal infections. The purpose of this review is to summarize our current understanding of the avian intestinal immune system and mucosal immune responses to enteric pathogens of economic importance in chickens such as *Eimeria*, *Cryptosporidium* and *Salmonella*.

INTRODUCTION

Host responses to enteric microbes are complex and involve many facets of cellular as well as humoral immune mechanisms. Many intestinal infections in poultry such as coccidiosis, salmonellosis, and cryptosporidiosis represent economically important diseases (reviewed in 22). Coccidiosis and cryptosporidiosis are caused by intracellular protozoan parasites which seriously impair the growth and feed utilization of livestock and poultry. In view of the complex life cycle of the coccidian parasites, it should not be surprising that host immune responses to these parasites are also complex. Following coccidial and cryptosporidial infections, both antibody and cell-mediated immune responses are activated, although cell-mediated immunity plays a major role in disease resistance (reviewed in 25, 27). Salmonellosis is an important food borne disease and the widespread occurrence of this disease in livestock and poultry challenges the most stringent measures of food harvesting, processing, monitoring, and control (36). Unlike many other infections of poultry, the primary target tissue for coccidia, *Cryptosporidium* and *Salmonella* is the intestinal tissue. Thus, understanding the immune system-microbial interactions in the gut leading to the elimination of enteric pathogens is crucial for the design of new approaches for controlling these infections.

GUT-ASSOCIATED IMMUNE SYSTEM

In chickens as in mammals, there exists a separate mucosal immune system that exhibits a number of unique features (reviewed in 22). The gut-associated lymphoid tissues (GALT) represent a component of the mucosa-associated lymphoid tissues (MALT) which also include the bronchial, salivary, nasopharyngeal, and genito-urinary lymphoid tissues. The MALT have evolved with specialized features that reflect their role as the first line of defense on mucosal surfaces. These include the presence of antigen-presenting, immunoregulatory, and effector cell types distinct from their counterparts in the systemic immune system.

Following oral administration of foreign antigens, activation of T-helper lymphocytes and IgA precursor B cells in GALT, especially in the Peyer's patches (PP), occurs. These cells then migrate to mucosal effector sites such as the lamina propria (LP) of the intestine and to the upper respiratory tract to mediate antigen-specific secretory IgA antibody responses. Activation of B and T cells in the GALT, followed by their migration to effector sites for the development of mucosal immune responses is termed the "common mucosal immune system" (CMIS) (30). CMIS consists of two separate but interconnected compartments; mucosal inductive sites which include the nasal-associated and gut-associated lymphoreticular tissues strategically located where they encounter environmental antigens and mucosal effector sites which include the LP of the intestine and the upper respiratory tract (reviewed in 30).

The GALT in chickens include organized lymphoid structures such as the bursa of Fabricius, cecal tonsils (CT), PP, Meckel's diverticulum, and lymphocyte aggregates scattered along the intraepithelium and LP of

the gastrointestinal tract. The bursa of Fabricius, a hollow oval sac located dorsally to the cloaca, is the central lymphoid organ for B-cell lymphopoiesis and lymphocyte maturation where antibody diversity is generated (reviewed in 34). The CT are discrete lymphoid nodules located at the proximal ends of the ceca near the ileocolonic junction (1). In the CT, both T and B cells are present in the germinal centers as well as plasma cells expressing surface IgM, IgG and IgA (15). The function of the CT is unknown, but active uptake of orally administered carbon particles has been shown, suggesting a role in antigen sampling (1).

The PP are lymphoid aggregates in the intestine which possess a morphologically distinct lymphoepithelium, with microfold (M) cells, follicles, a B cell-dependent subepithelial zone, a T cell-dependent central zone, and no goblet cells (1, 32). The PP represent the major inductive site for IgA responses to pathogenic microorganisms and ingested antigens in the gastrointestinal tract. The PP contain accessory cells such as macrophages (5 to 9%), functional dendritic cells and unique phagocytic cells such as M cells which possess numerous vacuoles reflecting active pinocytosis (3). Secretory IgA antibody is produced by plasma cells in the GALT and selectively transported through epithelial cells into external secretions.

In contrast to PP, CT lymphocytes consist mainly of $sIgG^+$ and $sIgM^+$ B-cells. As birds age, the intestinal lymphoid aggregates undergo involution such that by 20 weeks the lymphoid follicles become less distinct and fewer in number, and there appears to be a relative depopulation of the subepithelial zone in both the CT and PP. The Meckel's diverticulum is a remnant of the yolk on the small intestine, and usually persists as a discrete structure for the lifetime of the chicken. The exact function of this lymphoepithelial structure is not known but it contains germinal centers with B cells and macrophages (1).

Within the gastrointestinal mucosa, there are two anatomic compartments containing immune cells: the epithelium and the LP, which are morphologically separated by a basement membrane. Intestinal leukocytes from chickens contain 80% lymphocytes, 10-15% monocytes, approximately 5% other mononuclear cells, and less than 1% polymorphonuclear leukocytes and plasma cells (1). The intraepithelial cells in chickens are T lymphocytes expressing the CD3 polypeptides noncovalently associated with the γδ or the αβ chain receptor heterodimer of the antigen-specific T cell receptor (TCR) (5). With age, T-lymphocyte subpopulations change reflecting age-related maturation of the GALT (22, 24). The ratios of TCRγδ to TCRαβ cells in the epithelium and LP increase as chickens age. Jejunal $CD8^+$ IEL increase gradually until chickens are 4 to 6 weeks old and subsequently decline. $CD4^+$ cells represent a minor subpopulation among the IEL (21). In addition to lymphocytes, a third type of cell mediating intestinal immunity, called natural killer (NK) cells, exists. A recent study described a unique IEL subpopulation, termed TCR0 cells, showing cytoplasmic CD3 but lacking the surface TCR/CD3 complex. These cells were detected mainly in the epithelium where most expressed the CD8 antigen (6). Following infection, T and B cells initiate a series of antigen-specific and nonspecific responses involving antibodies, leukocytes, and locally produced cytokines. The roles of various components of the GALT in host defense against microbial infections has been studied extensively (4). Complex interactions between lymphocytes, epithelial cells, dendritic cells, and resident macrophages are involved in both secretory immunoglobulin and mucin production during the host defense process to generate a microenvironment incompatible with pathogen survival. Specific and nonspecific factors such as secretory immunoglobulins, anti-microbial intestinal peptides, and mucin influence the colonization of microbes.

Two basic types of lymphocytes are involved in antigen-specific responses: B lymphocytes that express surface immunoglobulin molecules with exquisite specificities for antigens, and T lymphocytes that recognize processed antigens on antigen presenting cells (APC). Upon binding of an antigen to B cells expressing surface Ig, cell division and clonal expansion ensue, and immunoglobulins with identical antigen specificity are secreted from the differentiated B cells. In contrast, T cells recognize small fragments of antigens that have been processed by APC only in conjunction with gene products encoded by major histocompatibility complex (MHC) genes. Recent studies have provided direct molecular evidence that antigenic peptides derived from protein antigens bind to MHC class I molecules (2). The interaction of the TCR-CD3 complex with self-MHC class I and II molecules and bound peptide antigen induces a cascade of events resulting in T-cell activation.

Cell-mediated immune responses include both antigen-specific and antigen non-specific activation of various cell populations, including T lymphocytes, NK cells, and macrophages. T lymphocytes are comprised of two functionally distinct subpopulations distinguishable by their surface phenotypes. Cytotoxic T lymphocytes (CTL, $CD8^+$) recognize foreign antigens in the context of MHC class I molecules, whereas T helper cells ($CD4^+$) recognize antigens in association with MHC class II

molecules. Although CTL activity has been demonstrated in the intestine of mammals, MHC-restricted IEL CTL activity has yet to be shown in chickens. It has been suggested that IEL NK cells are active in the first line of host defense because of their close proximity to the gut where a variety of antigenic substances are introduced (17). The observation that chicken intestinal IEL contain NK cells that mediate spontaneous cytotoxicity (19) suggests that NK cells may play an important role in local defense.

In chickens, IgA and IgM are the predominant immunoglobulins in external intestinal secretions. Secretory IgM, which is pentameric, is effective in elimination of microbes. However, several distinctive features are important for IgA to function as a secretory antibody. The major functions of secretory IgA (sIgA) include prevention of environmental antigen influx into internal body compartments, neutralization of viruses and microbial toxins, and prevention of adherence and colonization of mucosal surfaces by microbial pathogens. In this manner, microorganisms are susceptible to the natural cleaning functions of the mucosae. However, the role of secretory immunoglobulins is less clear in some poultry infections (17, 18, 31, 35). Despite the absence of immunoglobulins, agammaglobulinemic chickens are resistant to reinfection with coccidia (17). Therefore although the primary role of sIgA is to prevent invasion of microbes in the intestine, it is less certain whether sIgA limits the course of major infections once they are established.

INTESTINAL IMMUNE RESPONSES TO ENTERIC PATHOGENS

Coccidia - In chickens, a large percentage of the cellular infiltrate is composed $CD8^+$ lymphocytes, which are sometimes visible as large aggregates in the crypts and LP (38). Following primary infection with *E. acervulina*, most sporozoites were seen inside $CD8^+$ lymphocytes (37, 38), indicating that these cells are responsible for sporozoite transport. A significant number of sporozoites were also detected inside macrophages. $CD8^+$ cells are present in large numbers by 24 h following *Eimeria* infection (23, 38), and significantly more sporozoites are found in or next to $CD8^+$ cells in *E. acervulina*-immune chickens as compared with naive chickens. Two-color immunofluorescence analysis of duodenum IEL after infection with *E. acervulina* shows that at 24 h post-primary infection, many sporozoites are located predominantly within $CD8^+$ T cells and macrophages (38). Following secondary infection, there was an accumulation of sporozoites in $CD8^+$ cells, suggesting that sporozoites were unable to exit these cells to complete their journey to crypt epithelial cells (38). Furthermore, when $CD8^+$ cell-depleted chickens were infected with *E. acervulina* or *E. tenella*, there was, on average, a 55% decrease in oocyst production during a primary infection (39). Recently, a chicken monoclonal antibody which recognizes the coccidia conoid, an apical complex structure known to be involved in host cell invasion by *Toxoplasma gondii* (40), has been developed and shown to inhibit sporozoite invasion into host lymphocytes *in vitro*.

Animals infected with *Eimeria spp.* produce parasite-specific antibodies in both the circulation and mucosal secretions (13, 18, 31, 35). However, it is now clear that antibody mediated responses play a minor role in protection against coccidiosis. The importance of T cell immunity to coccidia has been well documented (reviewed in 25, 27). The number of duodenal IEL expressing the CD8 antigen increased following primary and secondary infections (26). Two-color immunofluorescence analysis of duodenum IEL at 10 days following challenge infection revealed that the majority of $CD8^+$ IEL coexpressed $TCR\alpha\beta$. Depletion of either $CD8^+$ cells or $TCR\alpha\beta^+$ cells resulted in substantial increases in oocyst production following challenge *E. acervulina* infection in chickens (39). These results suggest that variations in T cell subpopulations may reflect *Eimeria* infection-related changes in the GALT and that a significant increase in $TCR\alpha\beta^+CD8^+$ IEL may reflect enhanced acquired immune status.

Cytokines and lymphokines have been shown to influence the course of coccidial infections (13, 20). Limited information exists concerning the role of various lymphocyte subpopulations in cytokine production during avian coccidiosis due to unavailability of recombinant avian cytokines. Cell culture supernatant from concanavalin A (Con A)-stimulated lymphocytes inhibited the replication of *Eimeria* parasites in MDBK cell cultures and, when administered to chickens, reduced oocyst production following both *E. acervulina* and *E. tenella* infections (20). Strain differences in *Eimeria*-induced γ-interferon (IFN) production were observed (29). Sporozoites and merozoites of *E. tenella* induced production of a tumor necrosis factor (TNF)-like factor by normal peripheral blood derived macrophages *in vitro* (41). After infection with *E. acervulina*, expression of tumor growth factor (TGF)-β4 mRNAs increased in intraepithelial lymphocytes, suggesting a role for TGF-β4 in *Eimeria* infection (14).

NK cells are a population non-T, non-B, non-macrophage mononuclear cells that play an important role as a primary host defense mechanism against tumors, bacteria,

and viruses, as well as in the homeostasis of normal tissues. As with mammals, the chicken intestinal epithelium contains cells that can mediate NK activity (12). Increased NK cell activity occurs in the early stages of coccidia infection (19), suggesting a role for NK cells in the control of parasite proliferation. Further characterization of chicken NK cells will increase our understanding of their roles in mucosal and systemic immunosurveillance against infectious agents.

Cryptosporidia - Cryptosporidia are coccidian parasites that inhabit the microvillous border of epithelial surfaces. Among the 20 different *Cryptosporidium spp.* known, two species, *C. meleagridis* and *C. baileyi*, specifically infect birds. Avian cryptosporidiosis manifests itself as a respiratory, alimentary, or renal disease (9). Histopathological changes include hypertrophy and hyperplasia of infected epithelial surfaces. Infiltrates of macrophages, heterophils, lymphocytes, and plasma cells are usually present. Microscopic lesions in enteric infections consist of villous atrophy, villous fusion, crypt hyperplasia and infiltration of the LP with large mononuclear cells, lymphocytes, and plasma cells.

Immunocompetent hosts usually develop a self-limited, short-term, intestinal illness following oral exposure to *Cryptosporidium*, whereas most immunocompromised hosts develop a prolonged, life-threatening illness (8,9). Parasites have been found within the microvillous region of M-cells covering the PP, suggesting that M cells may initially bring cryptosporidia into close contact with the local immune system. Limited immunological studies of cryptosporidiosis have been carried out in chickens (10). The autoinfective nature of *Cryptosporidium*, unlike *Eimeria*, makes the development of resistance to this parasite difficult. The ability to develop immunity and to clear infection were shown to depend upon the host age and immune maturity (11). Chickens routinely develop an immune response to primary intestinal or respiratory infections and developed resistance to reinfection (11). Clearance of parasites from the intestine was accompanied by high titers of serum antibodies that recognized more than 20 distinct *C. baileyi* antigens, and the appearance of cell-mediated immunity to tuberculin, and oocyst and sporozoites antigens. A single infection with *C. baileyi* elicited protective immunity in broiler chickens. Although the immune mechanisms responsible for innate and acquired immunity to cryptosporidiosis are not understood, it is likely that locally produced factors as well as effector cells in the gut may be important. Furthermore, it is apparent that induction of host immunity is driven by a T lymphocyte dependent process, but the role of non-lymphocytic mediators of immunity, such as macrophages, NK cells, and polymorphonuclear leukocytes, needs to be carefully characterized. Since this parasite develops in the mucosal surface, studies should focus on intestinal immunity.

Salmonella - Domestic poultry constitutes the largest single reservoir of salmonellae in animal populations. The prevalence of *S. enteritidis* in poultry flocks is a great potential threat to both public health and the poultry industry. In chickens, the uptake of *S. enteritidis* and *S. thompson* by wandering macrophages across the cecal mucosa has been visualized ultrastructurally (33). *S. typhimurium* were recovered mostly from the CT and persisted until 33 days post infection (16). Infected chickens usually produce agglutinating type antibodies within 3 to 10 days post infection (36). As in mice, there is a lack of correlation between resistance to infection with the level of serum and biliary antibodies; resistance correlates with the development of CMI (7, 16). Compared to chickens receiving a killed *S. gallinarum* vaccine, immunization with a live vaccine produced significantly higher macrophage inhibition factor (MIF) at 10 and 21 DPI after challenge with live bacteria (7). Soluble factors are involved in mediating protective inflammatory responses following salmonella infection. Increased resistance to *S. enteritidis* in one day-old chickens was observed following the administration of supernatants from mitogen-stimulated lymphocytes (28). Significant increase in heterophils was associated with lymphokine-mediated increase in resistance to *Salmonella*. Future studies on the identification of protective bacterial antigens and protective soluble factors will lead to the development of immunological control strategies.

CONCLUSION

It is clear that intestinal immune responses to microbial pathogens involve the complex interplay of soluble factors, leukocytes, epithelial cells, endothelial cells, and other physiological factors of the gut-associated lymphoid tissues. It will undoubtedly be some time before a detailed description of immune mechanisms involved in protection against many enteric pathogens of poultry becomes clear. The GALT operates in an extremely complex milieu compared to lymphoid tissues at other sites, and a variety of nonspecific environmental factors are likely to affect the response to enteric pathogens. Furthermore, innate and acquired immunity to microbes depends upon host factors such as age, immune status and genetic background of the host. The denoted factors can significantly influence the effective operation of the immune system. Thus, understanding the immunological responses involved in the development of protective immunity against poultry diseases should precede the

development of vaccines. With increasing public health concerns of salmonellosis and other infections via contamination of poultry products, it is important that better control strategies be developed. Integration of protective cytokines and lymphokines in the vaccination program should be explored in view of the anti-microbial property of these factors. The advent of new molecular techniques to manipulate the genomes of various pathogens, and an enhanced understanding of interactions of the GALT with peripheral lymphoid organs, will soon enable new approaches to vaccination against enteric pathogens.

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PATHOPHYSIOLOGY OF ENTERIC INFECTIONS

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INTRODUCTION

The changes in intestinal structure and function caused by infections with viruses, bacteria or protozoa vary depending upon the pathogenetic mechanisms utilized by the microbe. They also vary depending upon the state of resistance of the host. In spite of the range of changes which occur there are some common pathophysiologic pathways which are useful in understanding and diagnosing enteric diseases. This review will give examples of some of these common pathways.

The intestinal epithelium consists mostly of absorptive cells and undifferentiated crypt epithelial cells. Absorptive cells are responsible for the digestive and absorptive functions of the intestine. They dominate the surface epithelium of the large intestine and the villous epithelium of the small intestine. Undifferentiated crypt cells are the proliferating compartment, they differentiate into absorptive cells and the other epithelial cells of the intestine. During differentiation to absorptive cells, crypt cells migrate onto the villi or the surface epithelium. Undifferentiated crypt epithelial cells are the principal site for secretion of electrolytes, water and immunoglobulin A.

HYPERSECRETION

In some diseases the principal change is disruption of the regulatory pathways which balance absorption and secretion of electrolytes and water. The disruption results in an accumulation of electrolytes and water in the intestinal lumen and secretory diarrhea. There may be little or no structural damage to the mucosa in such a secretory disease. The classical examples of secretory diarrhea are human cholera and enterotoxigenic *Escherichia coli* infections. In these diseases exotoxins produced by bacteria in the intestinal lumen act like hormone analogues to upregulate secretion. The regulatory changes occur in epithelial cells and also in the nerves and mesenchymal cells of the lamina propria, which are part of the extrinsic regulation of secretion and absorption by the intestinal epithelium.

One component of enterotoxin action is thought to be directly on epithelium to increase the concentration of cAMP or cGMP in absorptive and crypt epithelial cells. Increased cyclic nucleotide concentrations inhibit neutral NaCl entry from the intestinal lumen into absorptive cells. Other forms of NaCl entry (electrogenic, carrier mediated) into the cell are apparently not affected. However in crypt cells the increased cyclic nucleotide concentrations stimulate secretion of Cl- into the intestinal lumen. This combined effect of decreased neutral NaCl absorption and increased Cl- secretion is characteristic of several enterotoxins, draws water into the intestine and has come to be referred to as intestinal secretion or hypersecretion.

Direct effects on crypt and absorptive cells were initially thought to be the principal mode of enterotoxin action. However, recent and emerging evidence indicates that action through regulatory components extrinsic to epithelium probably account for most of the secretory response to the enterotoxins of *E. coli* and *Vibrio cholerae*, and in other enteric infections. For example, enterotoxins also stimulate the release of serotonin from the enterochromaffin (enteroendocrine) cells of the epithelium. Serotonin acts through the enteric nervous system to stimulate secretion (impaired NaCl entry into absorptive cells and Cl- secretion by undifferentiated crypt epithelial cells). Secretion is also stimulated by the release of serotonin from most cells during allergic reactions and by the release of prostaglandin from phagocytes and fibroblasts during the inflammatory process. I am unaware of a poultry disease which has been shown to be principally a secretory diarrhea. However, hypersecretion probably contributes to the pathophysiology of many enteric infections in poultry because of the stimulation of intestinal secretion by the inflammatory process and immune response.

MALABSORPTION

Some *E. coli* attach intimately to the surfaces of absorptive cells and efface microvilli (attaching/effacing *E. coli*). Because microvilli are the site for the enzymes responsible for digestion and absorption, such lesions can be expected to result in decreased digestion and absorption, which if extensive could result in

malabsorptive diarrhea. There is also an inflammatory component to such lesions. Some attaching/effacing *E. coli*, such as 0157:H7 strains associated with hemorrhagic colitis and the hemolytic uremic syndrome, produce cytolytic toxins called Shiga-like toxins. There is evidence that neutrophils cause some of the tissue damage and stimulate intestinal secretion during 0157:H7 *E. coli* infections. It seems probable that both malabsorptive and hypersecretory components contribute to the process. Attaching/effacing *E. coli* infections are reproduced by oral inoculation of newborn chickens with mammalian isolates of attaching/effacing *E. coli*. They have also been reported to occur naturally in chickens. The prevalence and economic importance (if any) of attaching/effacing *E. coli* infections in poultry is not known.

Many of the viruses associated with enteric disease have a predilection for absorptive cells. For example, rotaviruses and coronaviruses selectively replicate in and destroy absorptive cells. This results in partial or complete (depending on virulence and host resistance) atrophy of villi in the small intestine and mild or severe malabsorptive diarrhea. Rotaviruses and coronaviruses usually spare undifferentiated crypt epithelial cells. Crypt epithelium undergoes hyperplasia and will regenerate the villi in a matter of days.

Similar processes are involved following absorptive cell destruction by coccidia. However, different species of coccidia vary so widely in their tropism in terms of location in intestine (small or large) and cell type parasitized (absorptive cell, crypt cell, mesenchymal cell) that the overall processes are likely much more complex than those in response to cell destruction by rotaviruses and coronaviruses. Furthermore, the inflammatory responses stimulated by coccidia are more intense than those characteristic of rotaviruses and coronaviruses. Hypothetically one would expect inflammatory regulators of epithelial cell secretion (prostaglandin, serotonin) to complicate (stimulate hypersecretion) malabsorption due to absorptive cell loss much more in coccidiosis than in rotavirus infection. Prostaglandin induced secretion is an important component of *Cryptosporidium parvum* (a coccidian parasite) infection of the intestine of swine.

REGENERATION

Proliferation and regeneration of intestinal epithelium is under complex regulatory control. One component of control is feedback inhibition of crypt cell proliferation by the products of absorptive cells, such as transforming growth factor B. Microbial destruction of absorptive cells results in decreased feedback inhibition and thus allows hyperplasia of crypt epithelium and accelerates regeneration of villous epithelium. Intraepithelial lymphocytes (gamma delta T cells in mice) upregulate both proliferation and differentiation of intestinal epithelial cells. Presumably T cell infiltration into damaged epithelium during inflammation and allergic reactions also stimulates regeneration. Polyamines (putrescine and spermine) also upregulate epithelial cell proliferation in response to trophic hormones. It is suspected that polyamines produced by the enteric flora may also contribute to proliferation. In the normal animal there are marked changes in the kinetics of epithelial cell replacement and thus in regenerative capacity with age.

COMPENSATION

When absorptive cell damage is confined to the small intestine (as is frequently the case with rotavirus and coronavirus infections), the large intestine compensates for small bowel malabsorption during the period of regeneration. In mammals, compensation depends on microbial fermentation of residual carbohydrate to short chain fatty acids. These short chain fatty acids facilitate absorption of Na+ and water in the large intestine. The microflora and its fermentative capacity are not well developed in newborn animals, and thus their compensatory capacity is less than that of adults.

CONCLUSION

Knowledge of the structural and functional changes characteristic of enteric infections is useful in understanding and diagnosing such diseases. It is also potentially useful in treatment. Recognition that hypersecretion with most absorptive processes intact is the fundamental change in *V. cholerae* and enterotoxigenic *E. coli* infections, led to the development of oral fluid therapy. Oral fluid therapy is a major advance for human medicine and a significant one for veterinary medicine. Argenzio recently pointed out that recognition of the common pathways for intestinal secretion in many enteric infections opens the way for the rational development of drugs to inhibit neuro-endocrine - immune communication in the intestine, and for the use of glutamine to stimulate neutral NaCl absorption and epithelial regeneration during enteric infections.

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PATHOPHYSIOLOGY OF NONINFECTIOUS ENTERIC DISEASES

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INTRODUCTION

The avian digestive tract has several features that merit consideration in pathophysiology. Food must be ingested in a highly digestible manner or be sufficiently disrupted by softening and grinding in the crop, proventriculus and ventriculus (gizzard) (10). Chickens lack amylase in salivary secretions, but amylase and other enzymes occur in the crop due to reverse flow of digesta. Three reverse peristaltic cycles exist. Gizzard content may be refluxed back through the proventriculus and crop. If the gizzard is empty, ingesta can initially bypass the crop and go directly to the gizzard and then be returned. Duodenal content regularly refluxes into the gizzard, and colonic content moves by retrograde peristalsis into the cecum. Poorly digestible materials that cause offensive stimuli in the duodenum are prone to reflux into the gizzard and actually delay gastric emptying.

The duodenum is the principal site of nutrient digestion and absorption in the chicken and is dependent on gastric, pancreatic, and biliary secretions, functional brush border enzymes, and structural soundness (10). Factors causing rapid passage of digesta, altered pH, or decreased net absorption of water will impair digestion and absorption. In the chicken, ingesta can pass through the entire digestive tract in as short as 2.5 hours. One-half of the ingesta passes in 12 hours and virtually all completes passage in 24 hours. Undigested feed in the feces of poultry indicates of a loss in digestive efficiency.

During the first week, growth of the chicken gastrointestinal tract may be five-fold greater than the rest of the body (8). In the small intestine, villus length more than doubles in the first two weeks, which corresponds with increased responsiveness to various feed ingredients. Structural and functional maturation is not reached until 20 to 30 days of age (1). Gut-associated lymphoid tissue increases correspondingly, but is less so in germfree birds and in those fed antibiotics, indicating the importance of gut bacteria in stimulating the influx of lymphocytes. The overall size of the gastrointestinal tract increases when substances high in antinutrient factors are fed (4).

The intestine receives gastric (gizzard) contents with a pH of 3.5 to 4.5 which must be adjusted to a pH of 6 to 7 in order for digestive enzymes to function efficiently (10,11). This is accomplished largely by bicarbonate from the pancreas, the basic nature of bile salts, and the inherent buffering capability of the intestine. It can be speculated that peristaltic reflux would affect the balance of this process. Amino acid absorption is particularly sensitive to pH.

The avian digestive tract is a large secretory organ and osmolality is important to the balance of fluid. For each combined gram of water and food ingested, two grams of fluid are secreted from proventricular glands and intestinal crypts, and as pancreatic and biliary fluids. The net fluid absorption is completed when the digesta reaches the proximal half of the rectum. Material with high osmolality remaining after digestive attempts disrupts the balance of net absorption and results in loose droppings.

Solving a digestive problem requires consideration of noninfectious and infectious causes. As the following classification of noninfectious causes of digestive disease shows, some problems occur outside of the digestive tract. Many of the effects of noninfectious disease resemble infectious disease, and may involve them secondarily. This is sometimes understood and appreciated only in hindsight after considerable laboratory work.

REDUCTIONS IN THE ANTICIPATED NUTRIENT DENSITY OF FEEDSTUFFS

Technically, this isn't a digestive disease, it should be considered in the differential diagnosis of carcass yield and feed conversion problems. Drought and fungal damaged grains are among the causes. Mold growth uses the nutrients in the grain and decreases the energy, crude protein, and fat values. If not taken into consideration, the nutritional value of feed can be less than anticipated (9). Other issues include the amount of saturated vs. hydrolyzed fatty acids, predicted vs. actual amino acid concentrations, and damaged (overheated) or improperly processed ingredients such as soybean oil meal (26).

INTERFERENCE WITH PREHENSION AND SWALLOWING

External and internal factors can impair the desire or the ability to eat and swallow. Birds refuse to eat because of noxious stimuli in the feed. Feed refusal occurs with feeds contaminated with trichothecene mycotoxins which are produced by Fusarium and other fungi (17). People afflicted with fusariotoxicosis in the form of alimentary toxic aleukia noted that bread made from contaminated grain caused a burning sensation of the mouth, tongue, throat, and stomach (42). Some trichothecenes are caustic to the upper digestive mucosa and further impair the process of ingestion and swallowing. Inappetence and inanition are common to many systemic diseases.

ANTINUTRITIONAL FACTORS IN FEED INGREDIENTS

Many grains used in animal feeds have components that are either indigestible or act as specific or general blockers of the digestion of other nutrients (13,39). Some are storage polysaccharides and proteins that are inaccessible to endogenous enzymes. The presence of an endogenous enzyme needed to digest a factor may be age-dependent; for example, the enzyme may occur only in older animals. The digestibility limitations of grains are determined by the amount of fiber (fibrillar polysaccharides), matrix polysaccharides, and encrusting material. Specific compounds include beta-glucans, arabinoxylans, glucosinolates, pectins, oligosaccharides, cellulose, lignin, tannins, protease inhibitors, and phytate. These occur to varying degrees in barley, wheat, rye, triticale, sorghum, uncooked soybean meal, rapeseed meal, sunflower meal and cottonseed meal.

Among the adverse consequences are impedance of digestion and nutrient absorption, altered passage rate of digesta, increased microbial activity in the small intestine, and altered texture and color of the feces which become sticky. Beta-glucans found chiefly in barley but also wheat, triticale and rye, cause the intestinal content to gel. This interferes with the digestive action of endogenous enzymes and bile acids and with the absorption of digested nutrients in the intestinal lumen (1). The microbial population changes in response to the increased fermentative load in the lower tract. An increase in opportunistic bacteria causes additional insult to the digestive tract and increases the need for intestinal antibiotics. The overall size of the digestive tract may enlarge (4). The feeding of supplemental enzymes capable of digesting these materials diminishes but may not completely resolve the adverse effects (2,29).

CHANGES IN THE AMOUNT OF A DIGESTIVE SECRETION

Gastrointestinal secretions are normally stimulated by digestive hormones such as gastrin and secretin, stimulation of the vagus nerve, and various drugs and toxins (10,11). Secretions may be increased or decreased depending on the presence or absence of the proper stimulus. Decreases may also occur by loss of functional parenchymal cells in a digestive organ.

Histamine causes the chief cells in the proventriculus to secrete hydrochloric acid which results in a net increase in total acid secreted by the proventriculus and a decrease in the pH of gastric content (10). The histamine response is dose-related to the point of physiologic exhaustion. Exogenous sources of histamine and biogenic amines with similar actions, such as gizzerosine (16), occur in poultry feeds by way of protein sources prepared from fish and other animal byproducts. The amines are formed when spoilage bacteria produce the biogenic amines from the metabolism of free amino acids (21). In general, the greater the degree of spoilage, the greater the amine concentration. Gizzerosine is a biogenic amine that forms from free histidine and histamine in overheated fish meal (28).

Excessive histamine stimulation causes the proventriculus to become dilated and flaccid. The gizzard lining becomes roughened and sometimes ulcerated, and the intestine is dilated with watery content (34). Gizzerosine causes gastric hemorrhaging, likely from the lymphoid tissues or from acid damage to the proventricular mucosa, and the birds may actually vomit blood. Impaired performance parameters indicate that the digestive process is affected. Lowered intestinal pH can start a chain of events leading to maldigestion. Low pH decreases the efficiency of enzymatic digestion of carbohydrates and the absorption of amino acids. This changes the osmolality of intestinal content by way of the abnormal molecules presented to the lower intestine, and has the potential to overload the fermentative capacity of the cecum.

The insecticide fenthion is an irreversible cholinesterase inhibitor that causes increased gastric acid secretion and decreases the gastric pH in chickens (24). Various mycotoxins and copper sulfate cause gastric lesions similar to histamine (14,17) but the mechanism of injury is not well understood. Weak organic acids are likely to be absorbed in the gastric mucosa (22). Aflatoxin impairs pancreatic and biliary secretions. Dietary aflatoxin causes malabsorption seen as steatorrhea and hypocarotenoidemia linked to decreased concentrations

of bile salts and pancreatic lipase, trypsin, amylase, and RNAse (31,32).

DEGENERATION AND NECROSIS OF CELLS ACTIVE IN DIGESTION AND ABSORPTION

This is a serious event in the digestive tract because a series of critical events must occur in order to regain function (30). The consequences vary from lethal injury to diminished performance in the form of yield, feed conversion, and egg production. The premature death of a cell requires replacement by another recently divided cell. If the necrotic cell was fully differentiated and highly specialized, such as a chief cell in the proventriculus or a villus enterocyte in the duodenum, the replacement cell lacks the specific functions of the lost cell. This is gained over time as the new cell differentiates and specializes. In the proventriculus, lost chief cells are often replaced by columnar epithelial cells which resemble those in the glandular duct. These cells likely do not have the capacity to secrete pepsinogen and acid, the normal products of the chief cell.

In the intestine, necrosis of villus enterocytes has immediate impact on digestion and absorption. The contribution to digestion from the brush border enzymes is lost, and there is a contracting and shortening of the villus yielding an overall reduction is absorptive surface. Absorption is dependent on mature functional cells for pinocytosis and for some materials, the tight junctions between them. Through regulation by chalones, the crypt epithelium begins to divide and the crypt becomes deeper due to hyperplasia of crypt epithelium. The villus may be repopulated but with cells that are not yet mature. Resumption of normal digestive and absorptive functions are dependent on the maturation process. The reduction in surface area not only decreases absorptive capacity, but the hyperplasia of crypt epithelium may increase the net secretory activity of the intestine. As described above, the lost digestive function increases the osmolality in the lower intestine and increases the fermentative load of the cecum, possibly beyond capacity. The final result is diarrhea and passage of incompletely digested material.

Some toxins impart a radiomimetic injury to the intestine, such as that caused by T-2 toxin and other trichothecenes. With T-2 toxin, necrosis first affects the cells at the tips of the villi, probably a result of the caustic injury, and then the rapidly dividing cells of the crypt as a result of radiomimetic action, likely through inhibition of protein synthesis and other metabolic pathways (19). The villus is impaired by increased loss of cells from the tip and the replacement process is temporarily interrupted by crypt necrosis.

Many toxins cause damage to cell membranes in the gut and elsewhere by the formation of free radicals that cause peroxidation of membrane lipids (35). This causes increased membrane permeability and loss of specialized membrane functions, and may cause death of the injured cell. Under many circumstances, the free radical exists only briefly and incites local injury. With lipids however free radicals form in chain reactions called lipid peroxidation, leading to the formation of aldehydes and additional free radicals. This results in widespread damage to DNA, enzymes, and structural proteins. Poultry that consume feeds containing oxidized (rancid) fat thus face an immediate challenge to digestion. Free radicals incite damage in the stomach, small intestine, pancreas, and liver (37). Poultry fed rancid fats have reductions in weight gain (5) and corresponding decrease in intestinal villus length and surface area (8). The effect can be neutralized by antioxidants such as vitamin E and ethoxyquin which scavenge free radicals but the vitamin may no longer be available to the bird.

SUPPRESSION OF THE DIGESTIVE TRACT IMMUNITY

In man, the digestive tract contains as much lymphoid tissue as the spleen and as many as 80% of the immunoglobulin producing cells in the body are in the intestinal mucosa. (3). Such a figure is not available for birds but it is likely a close estimate. Lymphoid tissue is scattered through the upper digestive mucosa, at the proventriculus-gizzard junction, in the duodenum, distal ileum and cecal tonsils (15). The lamina propria of the gastrointestinal mucosa is rich in lymphocytes. Birds differ from mammals in that there is not a continuing supply of B cells following bursal involution. This has implications for B cell renewal from post-bursal B cells (41) which would seemingly implicate a role for those resident in the digestive tract. An intact immune response is important for production-limiting protozoan, bacterial and viral diseases.

In poultry, digestive immunosuppression is caused by toxins and nutritional deficiencies. T-2 toxin, a trichothecence mycotoxin, causes dose-related necrosis and depletion of lymphocytic tissues in the digestive tract. (19,20). Although lymphocyte repopulation occurs, tissues such as cecal tonsil may have permanently depleted diffuse lymphocytic tissue. Germinal centers are reduced in number if a young bird is exposed when seeding of secondary lymphoid tissues occurs from the bursa (18,20). Many mycotoxins affect the immune system including aflatoxin (25), sterigmatocystin (36), ochratoxin (12,23), and uncharacterized toxins of *Penicillium citrinum* (33). Nutritional deficiencies of

vitamin E and selenium impair immune function in poultry (27) and can affect immunity to coccidiosis (6). Again, toxic interactions can undermine a nutritionally complete diet, as T-2 toxin decreases plasma vitamin E concentrations (7).

IMPAIRMENT OF DIGESTIVE TRACT CONNECTIVE TISSUES

Ducklings with deficiencies of vitamin E and selenium develop necrosis of smooth muscle in the gizzard and small intestine (38). Affected segments of bowel are dilated by fluid and gas in the small intestine and caseous cores in the cecum. Ochratoxin increases intestinal fragility of broiler chickens, possibly by impairing collagen formation (40). Fragile intestine is prone to breakage during processing which causes carcass contamination.

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DIAGNOSTIC APPROACH TO ENTERIC DISEASES

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INTRODUCTION

Accurate diagnosis of enteric infections requires knowledge of possible etiologies, quality samples, competent diagnostic support, and a relentless desire to solve difficult problems. A dedicated team approach is needed. Whether the problem is viral, bacterial, parasitic, fungal or nutritional in origin, or a combination of etiologic factors, the same approach is required. The key is to see the forest as well as the trees.

METHODS AND SAMPLES

An accurate history is the starting point. This should include clinical signs, age, vaccinations, feed, drug treatment, environment, management factors, and all pertinent events leading to the outbreak. The general appearance and condition of the birds and their environment is a high priority. Representative birds for the condition should be selected for evaluation. The birds should be examined grossly for external signs of disease. Blood samples should be collected for blood smears, hematocrits, and for serologic testing. Following euthanasia a complete necropsy examination is conducted. Samples for bacterial culturing should be aseptically collected from selected tissues. Tissue samples should be collected for histopathological evaluation. Samples of gut contents should also be collected and frozen for analysis. It is better to collect tissues and discard them if they are later determined to be unnecessary or unimportant to the diagnosis.

BACTERIAL AGENTS

Analysis of the samples should begin as soon as possible. For bacterial cultures samples should be inoculated onto appropriate media. If *Salmonella* organisms are suspected, selective enrichment media such as tetrathionate broth should be employed to enhance the success of an accurate diagnosis. Positive *Salmonella* samples should be submitted to a reference laboratory for serologic typing. *Clostridium perfringens,* the cause of necrotic enteritis, can be isolated under anaerobic incubation at 37°C on blood agar plates. Positive identification can be made by inoculation of differential media.

PARASITIC AGENTS

Coccidia, hexamita, histomonads, and trichomonads can be identified by making a wet mount smear of mucosal scrapings from various segments of the intestinal and cecal contents. The smears can be examined directly under the microscope for suspended oocysts and merozoites and stages undergoing development in epithelial cells.

Crytosporidia can be identified by flotation techniques for oocyst identification of fecal specimens. Histologic examination of tissues collected at necropsy reveal basophilic organisms with H&E stains that are intimately associated with mucsal brush borders.

Ascarid larvae and capillaria can be identified in exudate and deep mucosal scrapings. Double-poled eggs can be seen in capillarids.

VIRAL AGENTS

Viral agents tend to be more difficult to diagnose than bacteria. This is due to the lack of specific high quality immunologic reagents available for use in most diagnostic laboratories, the poor *in vitro* growth characteristics, and the expertise and expense required to perform electron microscopic evaluation of specimens.

In chickens the viral agents reported include calicivirus, coronavirus-like particles, parvovirus, enterolike virus, reovirus, adenovirus, arena-like virus, togavirus-like agent, and rotaviruses. In turkeys the agents include adenovirus, coronavirus, astrovirus, reovirus, enterovirus, and rotaviruses.

Coronaviral enteritis of turkeys or Bluecomb disease can be diagnosed by isolation of the virus in specific-pathogen-free poults or in embryonating turkey eggs and subsequent identification by electron microscopy. Sections of intestinal tract may be used for direct fluorescent antibody tests if specific antiserum is available.

Rotavirus Type A can be isolated in a continuous line of fetal rhesus monkey kidney cells (MA104). The most common technique used to identify rotavirus infection in feces or intestinal contents is by direct electron microscopy. If specific antiserum is available, different serogroups of rotaviruses can be distinguished. Detection of rotavirus RNA in intestinal contents or feces is an alternative diagnostic method. Rotavirus RNA can be identified by the pattern of migration of the 11 genome segments.

Reoviruses have been isolated from a variety of tissues. Isolation of the virus can be done via inoculation of the yolk sac or chorioallantoic membrane. The virus also grows in primary chicken cell cultures. Direct electron microscopy or RNA analysis can be performed.

Astroviruses, enteroviruses, and the other enteric viruses can be identified by electron microscopy. The accuracy and success of the technique is increased if specific antiserum is available such that immune electron microscopy can be done.

CONCLUSION

The differential diagnosis of enteric disease must include cultures for enteropathogenic bacteria such as *Salmonella ssp.*, smears to demonstrate protozoa, and microscopic examination to rule out enteric viruses.

Alternative diagnostic techniques can be used in research laboratories. These include enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction, and nucleic acid probes to diagnose enteric infections. These techniques are not available for use in routine diagnostic laboratories.

Diagnostic accuracy will be enhanced when in vitro cultivation techniques and quality reagents are developed. Until that time we must be persistent to ensure accuracy in solving enteric disease problems.

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MANAGEMENT AND PREVENTION OF ENTERIC DISEASES OF CHICKENS

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INTRODUCTION

Enteric diseases in broiler and replacement chickens (pullets and broiler breeders) are basically under control in the United States. Control of these diseases comes at only moderate expense when viewed historically. Subclinical factors negatively affecting feed efficiency and growth rate represent the largest costs to the poultry industry. Clinical disease causing morbidity or mortality is an increasingly rare occurrence. The major enteric diseases in broilers or in replacement broiler breeders and trends of interest include:

COCCIDIOSIS

Broilers - Coccidiosis is a problem in that it is the enteric disease of broilers that we spend the most dollars to control. The polyether ionophores have the greatest market share of the domestic coccidiostats. Chemical coccidiostats are also used, often in shuttle programs with the ionophores. The annual domestic broiler market for coccidiostats (1995) was approximately 95 million dollars. In 1991 the broiler coccidiostat market was $105 million. In 1992 the broiler coccidiostat market experienced a peak at $110 million. Annual production has grown from 28 billion to over 35 billion live lbs. from 1991-1995. While broiler live lbs. have increased 25%, coccidiostat gross sales have declined 10% from 1991 and 13.5 % from the peak in 1992. The net result for the broiler industry is a lower expenditure per pound of meat for cocci control.

Within industry management systems, the coccidiostat arsenal available in the United States gives excellent control. There is generally a high level of satisfaction among broiler production veterinarians as regards efficacy of coccidiostats. Water activity in litter is generally lower than in the past due to nipple drinkers and better ventilation techniques. Lower water activity in litter may serve to lower the cocci challenge.

Cocci leakage philosophies vary greatly between broiler integrators. The frequency of treatment and coccidiostat changes are dependent upon the cocci leakage philosophy of the company. Coccidiosis in broilers is generally over treated at the service tech level unless carefully supervised. In my own company coccidiosis treatments are not in inventory at most locations because treatment is so rare. Potential coccidiosis problem sources include: feed intake at bird level, inadequate quality control in feed manufacture, litter moisture, and resistance (chemicals vs. ionophores).

Poultry integrators who emphasize pigmentation (yellow birds) of finished product practice a high level of cocci control with less immunity leakage. Dollars spent on carotenoid pigments in feedstuffs intensifies the need for birds to pigment fully. *E. maxima* strains in geographic areas have developed resistance to high pressure coccidiostat programs. Broilers are quite sensitive to pigmentation loss from *E. maxima.*

Broiler breeder replacements - Seventy to eighty percent of domestic broiler breeder replacement pullets receive a coccidiosis vaccine. This method has proven very effective in control of coccidiosis. Coccidiosis vaccine is often blamed for a lack of uniformity in replacement pullets. (Note: Coccidiosis vaccine is also available for use in broilers.) Delivery of coccidiosis vaccines involves one of these four techniques: water, feed, spray (ocular), or gel (Immunogel).

Amprolium is routinely given approximately 10 days after coccidiosis vaccine administration. Birds that do not receive a coccidiosis vaccine are given a coccidiostat during rearing for development of natural immunity.

NECROTIC ENTERITIS

Necrotic enteritis is of low incidence in the domestic broiler industry, but it has not gone away. Predisposing factors for necrotic enteritis include: inadequate cocci control, poor quality animal by-products, wheat and barley > 25% of diet, and (possibly) soil type.

Necrotic enteritis is usually responsive to antibiotics and cocci control measures. Antibiotics commonly used for necrotic enteritis include: Lincomycin, BMD (Bacitracin

Methylene Disalicylate), and Stafac (Virginiamycin).

To combat high litter counts of *Clostridium spp.* integrators may use soil and litter treatments in an attempt to change the microflora of houses. Products utilized include: salt, Poultry litter treatment (PLT - Sodium bisulfate), and elemental sulfur. Results are variable.

MUCOID ENTERITIS

Mucoid enteritis is a common finding on birds at postmortem. What is the cause? What does it cost in performance? What can we intelligently do about it?

Personal clinical observations suggest that mucoid enteritis is over diagnosed by veterinarians and service personnel in the United States. Often what is diagnosed as mucoid enteritis is not enteritis at all. Mucoid enteritis is a common sign seen with feed withdrawal or deprivation of feed intake. This phenomena may occur any time that birds are caught and hauled around for part of the day before post-mortem examination. Other factors affecting feed intake may give the same presentation, such as: leg problems, fever (air sac or osteomyelitis), ADR, meal time or feed clean up program, feed outages, etc.

What happens in feed withdrawal to cause this phenomena? The intestinal mucosa starts sloughing six hours after feed stops passing down the gastrointestinal tract. Keep in mind that a normal bird at catch may not have eaten for 3 or more hours and is just getting ready to feed again. Birds transported some distance to the diagnostic lab or posting session may present a gastrointestinal tract with sloughed intestinal villi debris and mucus to confuse the diagnostician. The gastrointestinal tract changes associated with feed withdrawal should be considered when evaluating enteritis problems. Another helpful observation is that bile in the gizzard at noticeable levels (green) is usually indicative of birds being off feed for greater than 12 hours. There is something in the gizzard 70-80% of the time after feed withdrawal. Litter is the most common content of the gizzard once a bird is off feed.

NON-SPECIFIC ENTERITIS, FEED PASSAGE, AND FLUSHING

Non-specific enteritis/feed passage is a common observation made by service personnel in the broiler industry. Determining an exact cause is difficult. No impact on performance is seen in the more frequent mild cases. In severe cases performance (weight and feed conversion) may suffer. Feed passage is more easily observed in the area under nipple drinkers where moisture may accumulate. In cases of mismanagement in the following areas, litter moisture is higher and feed passage complaints increase: drinkers (valve stem pressure too high or height too low), ventilation (inadequate mixing or static pressure), and heat.

Feed borne toxins are often blamed for enteritis/feed passage. Rarely is a definitive diagnosis for feed borne toxins made. Mycotoxins (trichothecene) in cereal grains and biogenic amines in rendered by-products are assumed culprits. There are hundreds of unidentified mycotoxins. Mycotoxins may occur in feedstuffs on a sporadic basis, but personally I consider mycotoxicosis a junk diagnosis unless there is proof. Diagnosticians rarely prove cause and effect with other than indicator mycotoxins and the action list is short. Mycotoxin prevention/action: QA of cereal grain ingredients, mold inhibitors (proprionate and other organic acids), farm feed bin and mill cleanup, cooler operation (moisture, negative shrink). Mold inhibitors have limited efficacy in that additional mold growth and toxin production is inhibited, but there is no effect on preformed mycotoxins. Mold inhibitors (proprionate and other organic acids) are routinely added to grain as it is unloaded at the feed mill by many integrators. Rendered by-product with high levels of biogenic amines can cause feed passage and poor performance. Biogenic amines prevention/action: know supplier, QA of incoming by-products.

Feed passage and flushing vernacular/definitions: Feed passage (may be mild, moderate or heavy) is evidenced by the degree of feed particles successfully escaping the gastrointestinal tract and maintaining the appearance of feed. Customarily this is more evident as birds are put on withdrawal feeds. Withdrawal feeds are lower in protein and will usually be more yellow than starter or finisher as the percentage of cereal grain in the withdrawal is higher than other feeds. Flushing suggests that chickens are "blowing" feed and water. When birds are flushing, houses often"slick over" (become wet throughout the house).

A partial list of additional rule outs for non-specific enteritis, feed passage or flushing:

Genetics: Certain lines within breeds may have higher moisture in droppings. Performance of genetic lines with extra moisture in droppings is often excellent.

Ingredients/Formulation: undercooked bean meal, high sodium level in any ingredient (i.e. bakery by-product/meat products), sodium level

specification too high, high phosphorous level, high protein diet (potassium), Avatec (lasalocid).

Milling problems: improper mix (mixing profiles), microsystem malfunctions (mixing profiles), ingredients in wrong bins, ingredient scaling problems, improper grind (coarse), improper conditioning (temperatures too low), improper cooling (moisture too high).

Common remedies for feed passage or non-specific enteritis include: copper sulfate, iodine, chlorine, and vinegar. The term remedies is used because the efficacy of these products is dubious in light of the massive rule-out list that will not respond to any therapy other than removing the insult.

METAZOAN PARASITES

Roundworms (*Ascaridia galli)*: For all practical purposes, roundworms have disappeared from broilers in the United States. Occurrence is so rare that it is not worth mentioning. Anthelmintics are generally not used in broilers in this country. Roundworms occasionally occur in replacement pullets.

Tapeworms (*Raillietina cesticillus*): Tapeworms occur sporadically in replacement pullets and broilers and are generally considered insignificant. No treatment is usually offered.

Capillary worms (*Capillaria obsignata*): Capillary worms do not occur in broilers, but occasionally occur in replacement pullets and breeding hens. Caps are more of a problem in older housing. Methods to control capillary worms in the pullet house that allow a cap free bird to be moved to the hen house are most popular. Capillary worms may cause severe morbidity and production loss in hens in lay.

Cecal worms *(Heterakis gallinarum)*: Cecal worms are sometimes seen in breeder hens. Treatment is offered in some cases. Usually, unless the case is extreme, birds are allowed to build immunity to the parasite with no therapy.

PROVENTRICULITIS

Proventriculitis is primarily a problem when evident at processing. Contamination and resultant loss in line efficiency and yield are the areas of greatest concern. There are many theories and some excellent research in proventriculitis etiology. Proventriculitis contributing factors include: copper sulfate*, biogenic amines, infectious bursal disease*, reovirus, and mycotoxins (*greatest contributors).

VIRAL ENTERITIS

Reovirus, coronavirus, astrovirus, birnavirus, enterovirus, arenavirus, adenovirus, rotavirus: All of these viruses have been implicated as contributors to enteritis in chickens. Vaccines are available to immunize hens and broilers for reovirus and birnavirus (IBD). The true economic implications of these viruses in regards to enteritis are not fully understood. Generally viral enteritis is considered only a minor problem in the scheme of the modern broiler industry.

CONCLUSION

The buzz word for broilers in the next decade may become "microflora management." Microflora management springs forward as companies look to various interventions to control intestinal flora and ultimately the flora in the production facility. Where are the critical control points to successfully change or control broiler microflora and where can we get the greatest return in field performance and finished product quality? There are quite enough challenges in this area to stimulate our thinking for years to come.

MANAGEMENT AND PREVENTION OF ENTERIC DISEASE IN TURKEYS

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INTRODUCTION

The primary breeders have provided the turkey industry with birds possessing tremendous genetic growth potential. In order for birds to realize this potential, they must continually consume, digest, and absorb the nutrients required for growth. As this genetic potential increases, the margin for error in management decreases. Even a brief interruption in the consumption and utilization of nutrients can result in less than desired performance.

There is an ever-increasing list of infectious and noninfectious factors that can cause such an interruption. These factors can act alone or in concert to produce clinical syndromes in the brooder house ranging from "restlessness" to mortality within a flock of over 50%. Specific enteric diseases may still occur in turkeys, but most often enteric disease is caused by a combination of management problems and multiple infectious agents. Management practices aimed at preventing these situations or minimizing their effects when they do occur should be obvious, but are often overlooked.

DISEASE PREVENTION

There are three essential elements for the prevention of enteric disease in turkeys: 1) a good environment, 2) freedom from exposure to enteric pathogens, and 3) access to good quality feed and water at all times. The difficulty of maintaining these three elements simultaneously must not be underestimated.

Every organization has its own idea of what the best environmental conditions are for turkeys of a given age. The basic idea is to keep the turkeys comfortable and the litter dry. Ventilate and supply heat accordingly. Often, especially in cold weather, damp litter can result from poor ventilation and provide an excellent environment for the propagation of potential pathogens. Increased stocking densities can also result in wetter litter, more stress, and more opportunities for enteric disease. Another source of moisture in a house is the water used for cleaning and disinfecting. Clean and disinfect as soon as possible after the flock is moved to allow for adequate drying before new litter arrives. If the lumen of the intestinal tract is considered to be outside the bird, then it too is part of the bird's environment. Direct fed microbials have shown promise in helping to maintain good conditions there.

Traffic between farms, as well as between houses on a farm, provides a constant opportunity to spread potential pathogens. Basic biosecurity practices cannot be ignored. The more attention paid to the details, the less likely a flock is to become infected. The older birds on a multiple age unit should always be considered a source of infection for younger birds. Backtracking from older birds into the brooder house must be avoided. This is critical whether there are birds in the brooder house or not. A grower who walks through his big birds before setting up his brooder rings is asking for trouble.

Even with boots, coveralls, foot pans, etc., human nature often results in lapses in biosecurity. Single age management systems reduce the likelihood of such lapses. Off site brooding, separate contracts for brooding and growing, and other all-in, all-out strategies have proven effective in reducing enteric and other diseases. The economics of disease loss versus bird flow must be considered in such strategies.

Insect control is another critical aspect of enteric disease prevention. Poults will selectively eat darkling beetle larvae when available. These larvae have been shown to be at least mechanical vectors for enteric pathogens. The same is likely true for house fly larvae. Also, birds spending their time pecking around in the litter looking for larvae are obviously not eating the feed they need.

Turkeys are sensitive to changes in the quality and consistency of their feed. Factors such as rancid fat and mycotoxins can influence feed intake and utilization. Presenting poults with good crumbles one day and mash the next can result in decreased consumption or feed refusal. If feed reaches the pan as a mixture, poults will eat the crumble, leave the fines, and often look elsewhere for something at which to pick (litter, each other, etc.).

Feed pans must be kept at the proper height and feed lines in good working order. Shavings in the feed pan limit the birds' access to feed and can be a source of pathogens. Maintaining feed consumption is critical not only to provide nutrients for growth, but to deliver coccidiostats and antibiotics.

Drinker management is also important in preventing enteric disease. Access to good clean water is essential. If birds cannot get enough water, they will not eat enough feed to grow at the desired rate. Chlorinating, acidifying, or otherwise sanitizing the water can help eliminate another possible source of enteric pathogens. Bell drinkers must be kept adjusted to the proper height and water depth. Poorly adjusted drinkers are a common source of litter moisture that can contribute to enteric problems. Nipple drinkers must also be adjusted properly for height and pressure. In general, nipple drinkers are more sanitary and allow for drier litter conditions than bell drinkers. Recent experience has also shown a decreased incidence of enteric protozoa in birds on nipple drinkers.

DISEASE MANAGEMENT

Efforts to prevent enteric disease will occasionally (or more often) fail. One or more of the above factors will affect a flock and start a vicious cycle. Birds will stop eating feed and start eating litter. Along with the litter comes the possibility of coccidial oocysts, other protozoa, molds, bacteria, and viruses. The birds feel worse and become less likely to eat or even drink normally. If diarrhea develops, water consumption will increase and litter conditions will deteriorate. Meanwhile, the birds are not getting the nutrients and drugs in the feed. Growth is interrupted if not stopped permanently. Correction of any predisposing management problems must be accomplished and supportive therapy begun immediately if flock performance is to be salvaged.

The key to breaking this cycle is early recognition of the developing problem. Poults noisily running along the feed line may be the first indication. Birds acting chilled and huddling may be the next. Keeping the birds on feed is critical. This may be a simple matter of top dressing the feed with something to attract the birds' attention such as oyster shell, grit, cracked corn, etc. It may require dumping the feed pans and letting fresh feed flow in. Running the feed lines periodically may also stimulate feeding. Chilled birds will need more heat (5-10°F) to feel like moving to the feed pans and drinkers. Walking the birds will help move them in that direction. Grit is critical for poults that have begun eating litter if they are to have any hope of passing that material and resuming feed consumption. A poult with a litter impacted gizzard is doomed.

These situations are seldom the result of a single agent that can be treated specifically. Most often, there are viral as well as bacterial and/or protozoal agents involved. The viral components cannot be treated, but vitamins and electrolytes may help offset their effects on the gut. Antibiotics including neomycin, lincomycin, penicillin, and bacitracin have been at least partially effective in reducing the impact of this enteric disease cycle. Sarafloxacin has also proven effective in minimizing the effect of the bacterial component. Treatment of protozoa other than coccidia provides variable results. Even when mortality is curtailed, affected birds' growth will be retarded.

Cleanup following an affected flock is critical. Again, as with general biosecurity, the further cleanup and disinfection is taken, the less the chances of the next flock becoming infected. All litter must be removed, including sweeping the floors, and spread as remotely as possible from other poultry. Ideally, the litter should be allowed to go through a heat before spreading. A thorough wash down and disinfection should follow (formaldehyde is preferred). As previously mentioned, it is essential at this point to allow ample time for drying before new litter is placed in the house. Moisture left in the house will increase litter moisture even before poults arrive.

CONCLUSION

In summary, efforts to prevent enteric disease must focus on:

1) limiting exposure to infectious agents through biosecurity,
2) ventilation and heat to maintain bird comfort and litter conditions,
3) proper adjustment of feed and drinker systems, and
4) feed quality and consistency.

Managing enteric disease that occurs depends on:

1) early recognition of the problem,
2) supportive care (vitamins, electrolytes, supplemental heat),
3) maintaining feed consumption,
4) treating specific agents when possible, and
5) thorough cleanup and disinfection after an affected flock.

COMPETITIVE EXCLUSION IN POULTRY PRODUCTION

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SUMMARY

Competitive exclusion (CE) or the "Nurmi concept", in combination with conventional hygienic measures, has been shown to be very effective as a preventive measure against *Salmonella* infection in poultry.

The main application of CE is in newly hatched chicks and turkey poults, but it can also be used in older birds after an oral antibiotic treatment.

There are three main ways of giving CE treatment to newly-hatched chicks: individually, in the first drinking water or by spraying. The last two methods are suitable for dosing birds under field conditions.

In addition to *Salmonella* the concept has been shown to be effective against pathogenic *E. coli*, *Yersinia*, *Campylobacter* and *Clostridium perfringens*, and claims have also been made that CE treatment enhances the growth and lowers the mortality of birds.

The mechanism of CE is not fully elucidated. The two most often cited in the microbial control of *Salmonella* in poultry are: production of volatile fatty acids in the ceca, and occupation of sites on the mucosa.

Two CE products are commercially available. They are more or less selected mixed cultures derived from the cecal contents of healthy adult chickens.

INTRODUCTION

Poultry has long been known to constitute a significant reservoir and source of human salmonellosis. This has been tracked to our modern hatching and rearing methods, which separate the generations from each other leaving the hatchlings vulnerable to invading pathogens.

Even if greater emphasis has been given to hygiene control in production and processing, problems with pathogen contamination have persisted. Outbreaks and even epidemics of human illness from poultry are all too common now in many countries (5,17).

Mature and intact intestinal microflora constitutes one of the most important defense lines against invading pathogens. Any procedures that delay its development in the young or disturbs it in adults can help the pathogens to survive and proliferate.

Adult chicks as well as turkeys are resistant to infections with food-poisoning salmonellae and other enteropathogens mainly because of the rich intestinal microflora. Newly hatched chicks and turkey poults, on the other hand, are susceptible to a variety of enteric diseases because their intestinal flora has not yet developed.

The intestinal microflora of the chick changes with age. In commercially reared birds, bacteria belonging to only a few genera appear in the gut of chicks during the first days of life. The native adult flora becomes established in the small intestine within the first two weeks (9,10), but it takes over four weeks to develop in the caeca (6,58,82). From this point of view it is easy to understand that such chicks become very susceptible to *e.g. Salmonella* colonization.

One of the safest and most useful means of controlling *Salmonella* infection in poultry (in combination with conventional hygienic measures), and one which has already been applied to commercial poultry flocks in several countries, is microbial colonization control. This process is known as competitive exclusion (CE), or the "Nurmi concept" (74). The concept may be defined as: "Early establishment of an adult intestinal microflora to prevent colonization by enteropathogens."

HISTORY OF CE

The idea that the natural resistance of chicks to *Salmonella* infection develops with the establishment of an adult-type or mature intestinal microflora was first mentioned by Milner and Shaffer in 1952 (63), but was not investigated on a world-wide basis until the study of Nurmi and Rantala (68) had been published more than 20 years later.

The "competitive exclusion" of one type of bacterium by other types was used as a term for the first time by Greenberg in 1969 (39). He claimed that competitive exclusion of *S. typhimurium* from maggots of blow flies was so effective that the organism survived in the gut only if the normal microflora was simplified or eliminated. A similar phenomenon had been demonstrated earlier in higher animals (56). "Colonization resistance," a term synonymous with CE, was made up by van der Waaij *et al.* (114) when studying the intestinal populations in mice. The CE concept was applied to domestic chicken for the first time by Nurmi and Rantala (68), but the term "competitive exclusion" was not used in relation to poultry until the work of Lloyd *et al.* (55).

DEVELOPMENT OF CE PREPARATIONS

Undefined treatment material - Nurmi and Rantala (68) used material from the crop and intestinal tract of an adult chicken to protect newly-hatched chicks against a challenge with *S. infantis*. Later, the same group demonstrated a similar kind of protection using an anaerobic broth culture of intestinal contents from an adult fowl (76,78). The results of these studies have been confirmed by several research groups around the world and reviewed by Pivnick and Nurmi (74), Schleifer (85), Mead and Impey (60), Schneitz (87) and Stavric and D'aoust (108).

Due to differences in experimental procedures a recommendation to standardize the chicken assay method used to evaluate different CE preparations was made by Mead *et al.* (59).

Pure-culture preparations - During the last nearly twenty years numerous attempts have been made to use a selection of pure cultures for protective purposes, but results, so far, have indicated that pure cultures are very unstable during storage and/or manipulation on laboratory media (106,108).

All acceptable pure culture preparations described to date have one feature in common: they give good, or relatively good protection for a short period and then they tend to lose their efficacy (106). Any unambiguous explanations for this do not exist, but the use of artificial laboratory media in isolation, cultivation and storage surely alter bacterial physiology.

Commercial CE products - The only commercially available CE products today are BROILACT® and AVIGUARD and the next one to come is CF-3. They all are undefined (more or less) selected mixed cultures derived from ceca of adult chickens.

BROILACT® is the first commercial CE product and was developed by Orion Corporation in Finland. It was launched in Finland and Sweden in 1987 and, until 1994, it was sold in liquid form. After that, the lyophilized product substituted the original preparation. BROILACT® is a well characterized mixture of chicken intestinal bacteria. Thirty-two different pure cultures have been isolated from BROILACT®. These include 22 different strictly anaerobic rods and cocci, representing 5 genera, and 10 different facultatively anaerobic rods and cocci, representing 3 genera. BROILACT® is entirely free from spore-formers. Two nonselective and 11 selective media were used to isolate the strictly anaerobic strains from BROILACT®, and two nonselective and two selective media were used to isolate the facultatively anaerobic strains (unpublished data).

An ability to associate with the intestinal epithelial surface is a common characteristic of microbes that colonize the gastrointestinal tract (81,91). Competition for adherence sites on the mucosa is one of the suggested mechanisms of competitive exclusion. BROILACT® is based on this idea (70,87), and has proven to give good protection not only against *S. infantis* (86,88,90), which is the most common *Salmonella* serotype in Finland, but also against *S. kedougou* (92), *S. enteritidis* PT4, and *S. typhimurium*(15,19,61,66,86). It has also been shown to be efficacious in protecting newly-hatched turkey poults against *Salmonella* colonization (89).

The *Salmonella* reducing effect of BROILACT® has been shown in the field, and also in those flocks that were *Salmonella* positive in the hatchery (16).

BROILACT® given twice after antibiotic treatment to *S. Enteritidis* PT4- and/or *S. typhimurium*-infected replacement pullets completely prevented re-excretion of salmonellae (48,53,113).

BROILACT® has been shown to be effective against pathogenic *E. coli* (41) and *Campylobacter* (16). Additionally, it has been shown to decrease mortality due to necrotic enteritis and hepatitis, and reduce the counts of *C. perfringens*, which is one of the causative factors in necrotic enteritis (34). The results of field studies also indicate improvement in bird performance (16).

AVIGUARD is a lyophilized mixed culture developed by Life-Care Products Ltd. in UK, but is now owned and sold by Bayer Ag. It was launched in 1993. AVIGUARD is an unselected culture derived from the whole cecum contents of an adult SPF (Specific Pathogen Free) chicken. No published data is available concerning the efficacy or use of AVIGUARD.

Other CE preparations - CF-3 is a mixed culture developed using a continuous-flow (CF) culture system to select a population of facultatively and strictly anaerobic bacteria from a homogenate of cecal tissues and contents prepared from 10-wk-old broiler chickens (25,64). It has been shown to reduce *Salmonella* colonization in laboratory trials and in the field, and to improve bird performance (25,26).

Milk Specialities Co. is developing a commercial version of CF-3 (18). Twenty-nine different pure cultures have been isolated from CF-3. These include 14 strictly anaerobic rods and cocci, representing 7 genera, and 15 facultatively anaerobic rods and cocci, also representing 7 genera. One nonselective medium was used to isolate the strictly anaerobic strains from CF-3, and one nonselective and one selective medium were used to isolate the facultatively anaerobic strains (26).

MCE (mucosal competitive exclusion) preparation was developed by Stern *et al.* (111), and has been shown to be effective for the control of *Salmonella* (14) and to some extent also *Campylobacter* (110). The MCE preparation is an undefined composition prepared from scrapings or a piece of washed ceca by incubating in an anaerobic culture medium (111).

Very few detailed facts have been reported concerning *L. reuteri* (112), its use as a CE preparation in poultry, and its ability to prevent colonization of poultry intestines with enteropathogens. However, claims have been made that *in ovo* colonization of the gastrointestinal tract with *L. reuteri* inhibits hatchling mortality caused by salmonellae (32).

Safety requirements for undefined CE preparations - The official requirements or criteria applied for the current undefined preparations are (67):

1. Healthy donor bird from a health-monitored flock (preferably specific pathogen free (SPF) birds. This includes both ante and post mortem examination of the donor bird.
2. Good laboratory and manufacturing practices (GLP and GMP) adopted throughout the production.
3. Meticulous examination of primary inocula for human and poultry pathogens in laboratories certified by licensing authorities.

Additional or supportive guarantees for the safety of an undefined CE product:

1. Low incidence of contagious diseases in the country where the CE preparation is produced.
2. Series of consecutive cultivation steps in manufacture which comprise a total dilution of at least one per one hundred million.
3. Media used in propagation do not support the proliferation of mycoplasma or viruses.
4. Careful quality control of the composition of the final product batches using indicator organisms.

ADMINISTRATION OF CE PRODUCTS

CE treatment is normally given to newly-hatched chicks or turkey poults as soon as possible after hatch in the hatchery or on the farm. It can also be given to older birds after therapeutic doses of antibiotics to regenerate the intestinal microflora.

There are three main ways of giving BROILACT® to newly-hatched chicks: individually, in the first drinking water or by spraying. The last two methods are suitable for dosing birds under field conditions. Individual administration is restricted to treatment of very valuable elite stock where the number of birds to be treated is relatively low.

The usual way of administering CE preparations in the field has been via the first drinking water. Successful administration via the first drinking water in the field was done in Sweden by Wierup *et al.* (120, 121). However, this method is not always optimal. Sometimes some of the chicks refuse to drink and the CE preparation spreads unevenly among the flock (90). The viability of the anaerobic organisms shows a rapid decline especially in chlorinated water, and the culture may lose its efficacy before all the chicks have received an adequate dose (95). Chicks may also be exposed to salmonellae during transportation and even earlier if there is vertical transmission from infected breeders. In both cases, treatment on the farm via drinking water is too late (93).

The idea of using aerosols as a method of administering CE cultures was mentioned for the first time by Pivnick and Nurmi (74). Goren *et al.* (37,38) developed a spray application method to treat newly-hatched chicks in the hatchery, either in the hatchers themselves or in delivery boxes. Spray application of a CE preparation in the hatcher followed by drinking water administration on the farm was described by Blankenship *et al.* (14) and shown to be effective in controlling salmonellae.

Spray application, either manual (88) or automated (86), enables an even spread of the treatment material without causing any observable adverse effects on the health or performance of the chickens during growout (25). The results of studies performed by several research groups and

the 5-year field experience in Finland show that spray application is an effective means of dosing newly hatched chicks already in the hatchery.

Johnson (53) described a successful field study in which replacement pullets infected with either *S. typhimurium* or *S. enteritidis* were treated first with antibiotics and, after moving them to a clean house, they were given two CE treatments in the drinking water. Two other studies have also shown that *S. enteritidis* PT4 can be eradicated from replacement pullets by administration of CE after antibiotic treatment (48,113).

The possibility of newly-hatched chicks becoming infected at a very early stage (*e.g.* because of dirty hatchery environment) has encouraged researchers to look for administration methods that enable the birds to be treated prior to hatch (29,30). Cox and Bailey (28) developed an *in ovo* administration method in which the CE treatment is inoculated into the egg, either into the air cell or into the amnion a few days before hatch. However, the use of a CE culture derived from the whole cecum contents of an adult bird, containing highly proteolytic organisms and abundant gas formers, resulted in low hatchability percentages when introduced into the air cell (31,28). When the CE culture was introduced into the amnion no birds hatched. Any effects on hatchability can be avoided by excluding proteolytic organisms and those that form abundant gas. Also, the timing of injection is crucial (unpublished data).

BENEFITS ACQUIRED BY USE OF CE

Studies performed in various countries prove that the CE concept applies to all serotypes capable of intestinal colonization (74,87,108). Treatment of commercial broiler flocks including millions of birds has shown that the concept is effective also in the field (16,38,121,122).

Turkey poults are fully protected with CE preparations from the same species (80,89,94) and Weinack *et al.* (117), Impey *et al.* (51) and Schneitz and Nuotio (89) showed that native chicken and turkey microfloras provided reciprocal protection in chicks and turkey poults.

Although the concept was originally devised to control *Salmonella* infections, it has been shown experimentally that CE treatment also protects chicks against pathogenic *Escherichia coli* (41,102,107,116,117,118), *Yersinia* (105), and *Campylobacter spp.* (2,16,103,104,110). It has also been shown that CE treatment decreases mortality due to necrotic enteritis and hepatitis and reduces the counts of *Clostridium perfringens*, which is one of the causative factors in necrotic enteritis (7,34,100).

Claims have also been made that CE treatment enhances the growth and lowers the mortality of birds. According to Goren *et al.* (37), an improvement in growth rate was observed in commercial broiler flocks sprayed with an undefined CE culture. Corrier *et al.* (25) reported an improvement in the efficiency of feed utilization in broiler flocks that were given CE treatment on the day of hatch. An improvement in bird performance in terms of higher bodyweight, better feed consumption, and lower feed conversation, in addition to lower mortality, was also obtained by Abu-Ruwaida *et al.* (1). Higher bodyweight and lower mortality were also noticed by Bolder *et al.*(16) in CE treated flocks.

MECHANISM OF CE

Microbial interactions and the mechanisms by which indigenous intestinal microorganisms inhibit colonization by invading pathogens have been thoroughly investigated over years. However, the mechanism of CE is still poorly understood. Neither do we know which bacteria are involved, though constantly effective mixed cultures have been propagated by excluding clostridia and facultatively anaerobic Gram-negative rods (unpublished results). Apparently, the only universally accepted fact concerning the mechanism of CE is that protection depends upon the administration of viable anaerobic bacteria.

Among the mechanisms by which one or more bacterial species may inhibit the proliferation or reduce the number of other bacterial species are (81):

1. Creation of a restrictive physiological environment.
2. Competition for bacterial receptor sites.
3. Elaboration of an antibiotic-like substance.
4. Depletion of or competition for essential substrates.

1. Volatile fatty acids (VFA), including acetic, propionic and butyric acids, which are produced by cecal anaerobes, are known to be inhibitory to salmonellae, especially in the undissociated state below pH 6.0 (62).

The importance of volatile fatty acids as part of the mechanism of CE has been claimed by several researchers (8,25,26,65). On the other hand, Seuna (93), Soerjadi *et al.* (102) and Stavric *et al.* (109) found that protection against an oral *Salmonella* challenge starts to become apparent only 1-2 hours after CE treatment. A similar observation was made by Mead *et al.* (61) when studying the efficacy of CE treatment to prevent transmission of *S. enteritidis* in delivery boxes. All these studies indicate that, initially, protection is predominantly

a physical phenomenon rather than one involving synthesis of VFA or any other metabolites.

2. Ability to adhere is important to bacteria in establishing or maintaining colonization on mucosal surfaces (83). The bacterial glycocalyx, which is considered to be any polysaccharide-containing component outside the cell wall (27), is thought to mediate adherence of protective bacteria to neighbor cells and to the intestinal epithelium of the chick. This forms a layer of cells which blocks receptor sites for *Salmonella* attachment (35,84,99).

3 and 4. The importance of competition for growth-limiting nutrients as a means of controlling microbial populations is difficult to evaluate because of inhibitory factors in the environment (81). Mechanisms 3 and 4 above have been studied in relation to pathogens other than *Salmonella* and host systems other than chicks, and their role in the avian alimentary tract has yet to be investigated.

In addition to the mechanisms suggested above, there is a multitude of host-dependent factors that interact with the normal intestinal microflora, and thus might influence the exclusion of pathogens. However, it is extremely unlikely that only one mechanism is involved in excluding invading pathogens from the intestine.

DISCUSSION

The most important measure to prevent carriage of food-borne pathogens in poultry is to improve hygiene in poultry production (73,11,20). Other measures are:

1. Competitive exclusion (67,87,108).
2. Vaccination (11,120,123).
3. Incorporation of organic acids in the feed (44,45,50).
4. Inclusion of fermentable carbohydrates *e.g.* mannose (71),lactose (22,23,24,72) or fructooligosaccharide (3) in the drinking water or in the feed.
5. Elimination of salmonellae from the gut with lytic bacteriophage (13).

Also, the impact of used litter on reducing *Salmonella* colonization in chicks has been studied (21).

Competitive exclusion and vaccination are two established methods to prevent the colonization of bird intestines by enteropathogens, especially *Salmonella.* Intervention with the proliferation and spread of enteropathogens at this early stage of the food production chain is probably the most important single action to ensure safer food to the consumer (67).

Because opposite results have been reported with the use of organic acids (43), mannose (52), and lactose (115), their use should be better explored and their potential value ascertained. The same is true for the use of fructooligosaccharide and the elimination of salmonellae with lytic bacteriophage. On the other hand, the efficacy of the anaerobic cecal cultures (3,22,42) and the continuous-flow cultures (23,24 65) used in all these studies was poor, which may explain the positive results gained by use of the sugars.

Factors that may increase *Salmonella* shedding are stress and disease. Newly-hatched chicks are more sensitive in this respect than older birds (36,54,101,118,119).

Also, the effects of sub-therapeutic levels of antibiotics on *Salmonella* colonization or shedding in chicks and turkeys (either CE treated or not), have been investigated, but the results are contradictory (4,12,40,47,49,57,69, 77,79,96,97,98,99). However, over the past twenty years, there has been increasing concern among scientists and public health authorities over the possibility that antibiotics used routinely at sub-therapeutic levels may contribute to the reservoir of resistant salmonellae and other resistant bacteria to which susceptible human beings can be exposed (33).

The timing of the CE treatment is also crucial; it should be given prior to the *Salmonella* challenge (93). However, evidence exists that CE treatment given after *Salmonella* challenge reduces the number of salmonellae in the chicken cecum (86) and the number of infected birds in a flock (16).

CE preparations have been used routinely in Finland since 1976 (75) and now more than 90% of Finnish broilers are given the treatment. This appears to have contributed significantly to the decline in *Salmonella* contamination of both flocks and carcasses (46).

In Sweden, the CE method has been used since 1981. A controlled epidemiological study, conducted by Wierup *et al.* (122) during a period when *Salmonella* was spread from contaminated feed, established the efficacy of CE treatment under field conditions. During 1981-1990, 179 flocks, involving 3.82 million chicks, were treated, and only one of these flocks was found subsequently to be contaminated with salmonellae (121). Now the use of CE treatment in Sweden is regulated by law so that two consecutive broiler or turkey flocks after each salmonella contaminated flock must be CE treated. The incidence of salmonella in poultry in Sweden is as low as in Finland.

The two commercial CE products are accepted and used in several countries today. However, reluctance of authorities to grant licences to undefined products is one reason for the slow penetration to the market (67). This may also result in attempts to introduce characterized CE cultures as defined ones.

The World Health Organization, WHO (123), has given guidelines of the use of vaccines and antimicrobials together with CE. Since CE cultures cannot be defined in the same manner as either a vaccine or a veterinary medical product, WHO recommends authorities to create a special product category called "normal gut flora". WHO also suggests that "normal gut flora" should be distinguished from live probiotics which are preparations of only one or a few strains of microorganisms, the primary purpose of which is to improve animal health and productivity.

There is evidence that CE treatment also improves the growth rate, gives higher body weight, better feed consumption and feed conversion, and reduces mortality (1,16,25,37). This has also been seen in numerous unpublished field studies, but it may be impossible to constantly gain positive results because of so many natural variables in the field.

CE products are successfully used in breeders in many countries, but if the claims of improved growth and better feed conversion by use of CE can be proven, there will be a financial incentive to use the method in broilers also. This, again, will certainly diminish the spread of poultry-borne pathogenic bacteria, especially *Salmonella*, to humans.

CONCLUSION

Competitive exclusion has been proven to be very effective as a preventive measure against *Salmonella* infections in poultry. However one must bear in mind that CE is an additional tool against *Salmonella*, but it does not replace all the other management methods which must be practiced. CE is a good approach to adopt where the husbandry is already good because even in these flocks the system can break down through contamination of feed or litter. Competitive exclusion improves resistance to contamination. The other possible benefits acquired from the use of CE are also of great importance, at least from the producer's point of view. This may encourage and motivate them to use the concept, and thus diminish the spread of poultry-borne pathogenic bacteria, especially *Salmonella*, to humans.

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VIRUSES AND MALABSORPTION SYNDROME IN CHICKENS - SOME PROBLEMS TO BE SOLVED

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INTRODUCTION

In the late 1970s, an apparently new and infectious disease of broiler chickens was described. The disease was seen virtually worldwide and was named after the main clinical signs and macroscopic findings observed in particular locations, *e.g.*, malabsorption syndrome, pale bird syndrome, runting stunting syndrome (RSS), infectious stunting syndrome, helicopter syndrome, etc. In all cases, poor growth and retarded feathering were observed, but these were accompanied by a bewildering array of inconsistently appearing signs and lesions such as increased mortality, diarrhoea, pancreatic atrophy, proventriculitis, rickets and other bone changes, thymic and bursal atrophy, etc. (reviewed in 6). The term RSS will be used in this article, because it most accurately reflects the consistent findings seen worldwide.

This variability in the presenting signs and lesions posed a number of questions. Were the syndromes described in different locations actually the same disease? If so, what was the fundamental nature of the disease? What was the explanation for the variability in lesions? In my opinion, the information necessary to provide answers to these questions has not been gathered. I believe that we were dealing with the same underlying disease worldwide. It is proposed that the consistent signs of poor growth and retarded feathering were caused by the same agent(s) in all locations, but that the inconsistently appearing signs and lesions were due to circumstances and secondary infection with pathogens peculiar to different locations, perhaps accompanied by immunosuppression. However, it is possible that infection with a number of agents, the identities of which were different in different locations, could produce the same non-specific results. It is now apparent that some of the lesions, *e.g.*, proventriculitis can occur independently of RSS (2). Others, *e.g.*, rickets and thymic atrophy, may be secondary to starvation.

POSSIBLE DISEASE MECHANISMS AND ETIOLOGIC AGENTS

How might an infectious agent produce poor growth and retarded feathering? Obvious mechanisms include:

(a) direct action on the digestive tract and associated organs; and/or
(b) direct action on endocrine glands controlling growth.

Given the benefit of hindsight, one is struck by the paucity in the literature of adequate histopathologic examinations for and descriptions of lesions in the digestive tracts of affected birds. Similarly, apart from results described by one group of European workers (7, 8), there is little information about possible effects on the endocrine system. Therefore, I believe that the disease was not adequately defined; there is no checklist of specific or semi-specific histopathologic or biochemical markers to refer to, and diagnosis in most cases was based on subjective assessment of the presence of non-specific clinical signs and gross lesions. Goodwin et al. (1) have recently proposed that RSS should be diagnosed when sicknesses attributable to small intestinal pathophysiological deficits cannot be linked to known nutritional deficiencies or enteric pathogens, and unique small intestinal microscopic lesions containing virus are found. This definition at least has the advantage of obliging other investigators to look for similar lesions; this may lead to improvement of the definition, and highlight differences between outbreaks in different locations which merit further investigation.

Failure to define a disease will obviously complicate attempts to determine its etiology. If we are unsure of the exact nature of the disease, how can we evaluate attempts to reproduce it experimentally?

There is good evidence that RSS is an infectious disease. Attempts to identify the etiologic agent(s) have centred around:

(a) attempts to isolate viruses and other microorganisms from affected birds in the field; and
(b) attempts to experimentally transmit the disease, coupled with virologic and microbiologic examination of material from experimentally infected birds.

Many viruses have been associated with RSS. These

include reoviruses, rotaviruses, parvoviruses, enterovirus-like viruses, togavirus-like particles, coronavirus-like viruses, adenoviruses and caliciviruses. While some of these produce a temporary retardation of growth in experimentally infected chickens, none of these viruses has been conclusively shown to cause RSS (reviewed in 6).

Virologic examinations have consisted largely of attempts to isolate viruses in cell cultures or chick embryos, from faeces or intestinal contents of affected birds, and direct electron microscopic examination of the same material.

It is now clear that virus isolation is not likely to yield useful results. Many of the most recently discovered avian viruses do not grow, or are non-cytopathic, in conventionally used cell culture systems. Furthermore, many samples from young broiler chickens contain reoviruses. These will quickly overgrow other viruses. Similarly, direct electron microscopy requires that viruses are present in large numbers in the sample and that their morphology is distinctive enough to allow them to be identified. Therefore, virologic examination of affected birds taken from the field is likely to be an unrewarding experience, unless we are attempting to verify the absence/presence of specific viruses. Furthermore, by the time that growth retardation becomes obvious in the field, the causative agent may no longer be readily detectable.

This may suggest that attempts to experimentally transmit the disease, coupled with examinations of experimentally infected birds, would be a better option. However, this approach is also fraught with difficulties. The first concerns the choice of experimental subjects. RSS is a disease of rapidly growing birds (2). Thus it may be inappropriate to use SPF Leghorn chicks in transmission trials, even though they should be fully susceptible microbiologically. Given that SPF broilers are not readily available, the only option is to use commercial broilers. However, there may be variations between batches of birds in terms of susceptibility and presence of extraneous viruses and other microorganisms. The choice of inoculum, route of inoculation and age at inoculation also need to be considered carefully. Most workers have used intestinal contents from field cases, administered orally to one-day-old chicks. However, it may be preferable to collect samples from younger, as yet unaffected, broilers in the field, and to use as inocula those samples from flocks which later succumb to disease, in the hope that they will contain higher titres of the etiologic agent(s).

This still leaves the major problem of deciding on the criteria that signify successful experimental transmission. Obviously growth retardation and poor feather development must be reproduced. Many workers have reproduced stunting, *ie.*, a temporary retardation of growth and poor feathering, but it is not clear how frequently runting, *ie.*, a permanent retardation of growth, has been induced. In some instances, this may be a reflection of the logistics of experimentally reproducing a phenomenon which occurs at a prevalence of about 5% in the naturally occurring disease. However, it obviously raises uncertainty about the success of the experiments. Ability to produce the variable signs and lesions peculiar to particular locations has to be assessed against their perceived significance.

If it appears that the disease has been successfully transmitted, this will need to be repeated to ensure reproducibility and material will be passaged through successive batches of experimentally infected chicks. This will provide an opportunity to investigate the sequential development of gross and microscopic lesions. The latter will provide a yardstick for comparison with the naturally occurring disease. On the same occasions, virologic and microbiologic examination can be undertaken, using virus isolation and direct electron microscopy. While these techniques have limitations, as discussed above, at least they will identify some of the viruses which are present, as well as those which do not appear to be present. Antisera to those viruses detected can be used for direct immunofluorescent staining of cryostat sections to determine whether specific viral antigens are associated with development of microscopic lesions. Similarly, antisera prepared against the whole experimental inoculum may reveal antigens not detected by antisera to known viruses. If these antigens are associated with lesions, they are clearly of interest and can be investigated further by thin section electron microscopic examination of lesioned tissues. Such a strategy enabled a group from this laboratory to identify an enterovirus-like virus in the faeces of 1 to 6-day-old broiler chickens from a flock which subsequently developed RSS. Attempts to serially passage this virus in avian cell cultures were unsuccessful, and were complicated by the presence of reovirus (5). Provided that the etiologic agent(s) in the inoculum are sufficiently antigenic and present to sufficiently high titre, this approach offers the possibility of associating the presence of specific antigens with the development of lesions. The same conjugated antisera can be used to monitor inoculated cell cultures and chick embryos for growth of non-cytopathic and non-embryo lethal viruses, and also to examine material from affected and unaffected flocks from the field.

It is possible that RSS is not caused by a single etiologic

agent. Kouwenhoven et al. (2) reported findings which suggested that both viruses and bacteria were involved.

CONTROL MEASURES

The nature of RSS has implications for its control. If it is an enteric, as opposed to a systemic, disease, the prospects for control through vaccination are not good. There are 2 possibilities:

(a) breeder vaccination with an inactivated, adjuvanted vaccine; and
(b) broiler vaccination with a live attenuated vaccine.

Given the high prevalence of enteric viral infections in the progeny of breeder flocks with maternal antibodies to these viruses, it is questionable whether vaccination of breeders would protect their progeny against enteric infections. Similarly, assuming the problems associated with identifying, culturing and attenuating the etiological agent(s) can be overcome, the success of broiler vaccination would depend on how quickly and effectively vaccination could induce immunity against an early field challenge.

To date, the disease has been controlled in Europe, mostly by thorough cleansing and disinfection of affected sites. It has now virtually disappeared from Northern Ireland and from a number of other countries. Identification of the management factors which appear to be associated with the highest and lowest risk, through the use of databases similar to that developed in this laboratory (3, 4), and the subsequent use of this information to change management practices, may offer a faster, more sustainable and more practicable approach to controlling RSS than vaccination.

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POULT ENTERITIS AND MORTALITY SYNDROME ("SPIKING MORTALITY"): AN ACUTE, TRANSMISSIBLE DISEASE OF UNKNOWN ETIOLOGY

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INTRODUCTION

Poult Enteritis and Mortality Syndrome (PEMS), otherwise known as "Spiking Mortality of Turkeys" (SMT) is a recently identified enteric disease of young turkeys that potentially may result in devastating production losses (Table 1). PEMS is a transmissible, infectious disease that generally affects turkeys between the ages of 7-28 days. The disease is seen primarily as a severe enteric disease with high mortality. PEMS-affected birds initially exhibit depression and severe diarrhea; they tend to stop eating and drinking, and subsequently die. Mortality in PEMS-affected flocks generally exceeds 1% for at least 3 consecutive days; total mortality typically exceeds 9% and may be as high as 50%. Birds examined at necropsy have pale, thin-walled and distended intestines suggestive of a severe enteric infection; birds also exhibit thymic atrophy, bursal atrophy, and bursal cores. Microscopic lesions in affected birds generally include severe crop mycosis and moderate to marked lymphoid depletion in spleen, bursa of Fabricius, and thymus. Flocks that have recovered from PEMS exhibit severe stunting, increased susceptibility to other diseases, increased time-to-market, and increased feed conversion. In addition, the lack of uniformity within affected flocks also causes significant problems at processing.

Outbreaks of PEMS were first observed in western North Carolina in 1991. Since that time the disease has also affected turkey production areas in eastern North Carolina, as well as central Indiana, central South Carolina and Georgia. Unconfirmed cases have been cited in New York and the Shenandoah Valley of Virginia.

Economic losses due to PEMS are known to be considerable. It has been estimated that this disease cost the turkey industry approximately $15 million in 1994. Total losses since the disease was first recognized in 1991 have been estimated to be approximately $25 million. It is estimated that approximately one-half million poults died as a result of PEMS in eastern North Carolina in the summer of 1994. While no detailed study of the economic losses that occur as a result of this disease have been done, data from one company (Table 1) indicates that losses are incurred as a result of markedly increased mortality, poor feed conversion and lower market weights.

Table 1. Poult Enteritis Mortality Syndrome (PEMS): Economic data obtained from one company with affected flocks.

	PEMS Flocks	**Average**	**Top 10 Flocks**
Livability	63.4%	76.6%	84.8%
Average Age	139 days	139 days	136 days
Average Weight	29.9 lb.	32.4 lb.	33.6 lb.
Average Daily Gain	0.215 lb.	0.233 lb.	0.247 lb.
Feed Conversion	2.90	2.74	2.63
Cost Difference	+0.0476	0.0000	-0.0228

ETIOLOGY

The etiology of PEMS presently is unknown. A large number of different infectious agents have been identified in PEMS-affected turkeys and associated as causes of the disease. Protozoa associated as causes include *Cryptosporidium spp.* and *Cochlosoma spp.* Bacterial agents include *Salmonella spp., E. coli, Clostridium spp., Campylobacter spp., Bacteroides spp.* and *Arizona spp.* Several different viruses have been identified and associated as causes including: reovirus, rotavirus types A and D, turkey enteroviruses, turkey coronavirus, turkey adenoviruses, alphaviruses, and infectious bursal disease virus type 2. The agents that are most commonly identified in PEMS-affected turkeys in North Carolina are rotavirus, reovirus, enterovirus, adenovirus, *Salmonella spp.* and *Cryptosporidium spp.*; and if we look hard enough it is not uncommon to find most of these in samples collected from individual turkeys in PEMS-affected flocks. Turkey coronavirus is detected only sporadically; however, this may be due to relatively insensitive diagnostic procedures that currently are being used to detect this virus.

Investigators at the University of Georgia have identified a type 2 infectious bursal disease virus in PEMS-affected turkeys (1). Infectious bursal disease virus type 2 previously has not been considered to be pathogenic for turkeys, but based on the type of disease that infectious bursal disease virus type 1 causes in chickens, the pathogenesis of this virus in turkeys needs to be further investigated. In 1991 we identified two alphaviruses--eastern equine encephalitis virus and Highlands J virus--as causes of egg drop in turkeys and identified these viruses in turkeys flocks experiencing PEMS-like disease (2). Experimental studies with these viruses demonstrated that they could produce a syndrome very much like what is seen in PEMS-affected birds (3), but based on serologic and epidemiologic studies we now know that these two viruses are not causes of this disease. It is also possible that other yet-to-be-identified infectious agents are present in PEMS-affected turkeys; identification of these putative agents may be hindered by the nature of the agent(s) (*e.g.*, resistant to laboratory propagation, indistinct electron microscopic morphology) and/or because we presently lack the appropriate diagnostic tools for identifying these agents.

EXPERIMENTAL STUDIES

When we first began working with this disease in 1991, we made several unsuccessful attempts to reproduce the disease in our facilities at the North Carolina State University College of Veterinary Medicine (NCSU/CVM). Typically, intestinal contents from PEMS-affected turkeys were collected in the field, brought back to the college, and stored at -70C until we could arrange for space and obtain young turkeys for experimental exposure. This was done several times and repeatedly we failed to produce mortality in experimentally-inoculated turkeys. This led us to believe in the years between 1991-1994 that the disease was caused by factors other than infectious agents. Considerable efforts were made to identify toxic agents that would explain the disease, but this was unsuccessful. In 1994, we demonstrated that the disease was transmissible, using sentinel turkeys, and the infectious nature of the disease was convincingly demonstrated. Dr. Tom Brown at the University of Georgia much earlier had demonstrated that the disease could be produced by placing young turkeys on litter from PEMS-affected flocks; however, our failure to reproduce the disease with intestinal contents suggested the possibility that toxic substances in PEMS litter contributed to Dr. Brown's successful reproduction of the disease.

The failure to produce the disease using frozen intestinal contents collected from PEMS-affected turkeys suggested that the causative agent was susceptible to inactivation by freezing. Several infectious agents including protozoa, *Mycoplasma*, and certain cell-associated viruses are known to be inactivated in this manner, but of these agents only *Cryptosporidium* had been associated as a cause of SMT. Thus, this suggested to us the possibility that *Cryptosporidium* was causing the disease and prompted an intense investigation of the pathogenesis of this agent for turkeys (4). Experimental inoculation of young turkeys with purified preparations of *Cryptosporidium* oocysts indicated that *Cryptosporidium*, by itself, could cause moderate growth depression but only minimal mortality. In addition, we found that intestinal contents from PEMS-affected turkeys, filtered to remove infectious agents other than viruses, also resulted in moderate growth depression with only minimal mortality. However, severe growth depression and high mortality compatible with PEMS was produced when young turkeys were inoculated with a combined inoculum containing *Cryptosporidium* oocysts and the intestinal filtrate. Interestingly, turkeys inoculated with both *Cryptosporidium* and the intestinal filtrate exhibited enhanced shedding of *Cryptosporidium* oocysts as compared with shedding in those birds inoculated with only *Cryptosporidium*. This study indicated that PEMS was caused by an interaction between two or more infectious agents; *Cryptosporidium* and an unidentified enteric virus (4). The study also suggested the possibility that the disease was caused by an infectious agent, most likely a virus, that causes

immune suppression and potentiates the pathogenesis of ordinarily innocuous enteric agents such as *Cryptosporidium*. Immune suppression in PEMS is supported by the presence of gross and microscopic damage in lymphoid organs of affected turkeys and the observation that turkeys in recovered flocks have an increased susceptibility to other diseases. Studies conducted by Dr. Muquarrab Quereshi at North Carolina State University Department of Poultry Science also support the presence of immune suppression in PEMS-affected turkeys.

Subsequent studies have examined *Cryptosporidium* in combination with a variety of turkey enteric viruses in the hope of identifying the virus that interacts with *Cryptosporidium* to produce PEMS (4). Turkey coronavirus, turkey enterovirus and turkey reovirus were prepared as purified preparations and used as inocula for young turkeys, alone and in combination with *Cryptosporidium*; however, when these viruses were inoculated into young turkeys, alone or in combination with *Cryptosporidium*, they produced only moderate growth depression and either minimal or no mortality. *Cryptosporidium* by itself produced moderate growth depression in inoculated turkeys, but no mortality. High mortality and severe growth depression consistent with PEMS could only be produced by inoculating turkeys with a mixture of *Cryptosporidium* and the bacteria-free filtrate prepared from droppings of PEMS-affected poults.

We are continuing the search for other viruses that might be present in intestinal contents of PEMS-affected turkeys. In recent months, we have identified three previously unrecognized viruses in PEMS-affected turkeys: a picornavirus-like virus, a group I avian adenovirus, and an unidentified 55-70 nm virus-like agent. The picornavirus-like virus morphologically resembles a previously described turkey enterovirus (5); however, recent studies indicate that these are two distinct viruses. The picornavirus-like virus differs antigenically from the turkey enterovirus, and unlike turkey enterovirus, the picornavirus-like virus can be propagated in embryonated chicken eggs. The group I avian adenovirus is antigenically distinct from previously described turkey adenoviruses (Northern Ireland types 1 and 2) and replicates in intestine, proventriculus, bursa of Fabricius, spleen, and thymus of turkeys, tissue sites that correspond to the sites of tissue damage observed in PEMS-affected turkeys. The unidentified 55-70 nm virus-like agent has been detected in intestinal contents of PEMS-affected turkeys by immune electron microscopy and in intestinal tissues by thin-section electron microscopy. Using immune-electron microscopy, 50-90 nm, "fringed" particles are detected in intestinal contents of PEMS-affected turkeys, and similar particles (55-70 nm) are found by thin-section electron microscopy within intracytoplasmic vesicles of intestinal epithelial cells. Attempts to cultivate this virus-like agent in the laboratory have not been successful to date; thus, definitive identification of this agent remains undetermined. Experimental challenge studies presently are underway to assess the pathogenicity of these viruses for young turkeys and to determine their role in PEMS.

Additional studies are underway at NCSU/CVM aimed at determining the mechanisms of transmission of PEMS and identifying methods for controlling the disease; however, the search for the agent(s) that cause this disease will continue to be the focus of our investigations. Based on the historical record with other infectious diseases, it is unlikely that effective control procedures will be developed until the cause of this disease is identified.

CONTROL

PEMS is believed to be transmitted primarily by the fecal-oral route; it does not appear to be vertically transmitted. As a consequence, good biosecurity should be useful for controlling spread from one farm to another and from one flock to another on the same farm. In SMT-affected flocks, aggressive management to keep birds eating, keep litter dry and provide a comfortable temperature can greatly influence the overall impact of the disease. Sweet feed containing molasses, whole rolled oats, powdered milk, and confectionery sprinkles can help keep affected poults eating. Dumping feed pans, cycling feed lines, and getting birds up and moving frequently may improve feeding activity. Small firm pellets and good crumbles with little fines may reduce mortality; high fines in feed and mash feeds have been associated with increased mortality. The use of electrolytes and vitamins, particularly B-vitamins and vitamins A and E, also should help decrease the impact of the disease on a flock. Recently, the use of sarafloxicin has been shown to dramatically reduce mortality; this is the first report of a possible specific treatment for PEMS and it suggests an important role for bacteria in the disease.

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