Emerging and Re-emerging Diseases

Symposium Sponsored by the
American Association of Avian Pathologists

Symposium Program

Sunday July 25, 2004
Philadelphia, Pennsylvania
47th Annual Meeting AAAP/AVMA

AAAP Symposium 2004

Emerging and Re-emerging Diseases

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Acknowledgments: The Committee on Toxic, Infectious, Miscellaneous and Emerging Diseases thanks the AVMA and AAAP for financial support of this symposium and the printing of this program. Special thanks to the AAAP board members and Dr. John Glisson for their assistance in the organization of the symposium.

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Schedule

Session Moderator: Dr. H. L. Shivaprasad

8:00 AM	Dr. H. L. Shivaprasad – Welcome & Introduction.
8:10 AM	Dr. Corrie Brown – Emerging and Re-emerging Diseases - National and Global Perspectives.
8:50 AM	Dr. David Suarez – Bird Flu.
9:30 AM	Dr. Dave Cavanagh – What is New with Coronavirus?
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Session Moderator: Dr. William Hewat

10:30 AM	Dr. Kenton Kreager – Emerging Diseases and Conditions in Layers.
10:50 AM	Dr. Robert Owen – Emerging Diseases and Conditions in Broilers.
11:10 AM	Dr. Steven Clark – Emerging Diseases and conditions in Turkeys
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Session Moderator: Dr. Scott Fitzgerald

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1:20 PM	Dr. Aly Fadly – Retroviruses and Marek's Disease Virus.
1:50 PM	Dr. Roy Curtiss III – Emerging Bacterial Diseases.
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Emerging and Re-emerging Diseases – National and Global Perspectives

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The term "emerging disease" has a fairly broad definition and in general, encompasses any one of three disease situations – a known agent appearing in a new geographic area, a known agent or its close relatives occurring in a hitherto unsusceptible species, and a previously unknown agent detected for the first time. The last two decades have witnessed a steady parade of emerging disease problems, and even the rate at which new disorders are occurring is escalating. Basically, emerging diseases have created a new kaleidoscopic lens through which we view the world. In the words of the great American baseball player, Yogi Berra, "The future ain't what it used to be."

The two unrelenting forces of population growth and globalization continue to combine synergistically, allowing for possibilities for agents to move from comfortable domains into new unexplored niches. As articulated in a recent National Academy of Science report, we are creating the microbial equivalent of "a perfect storm". However, unlike a major climatic event, where various meteorological forces converge to result in a cataclysmic tempest, this microbial perfect storm will not subside. There will be no calm after the epidemic, rather the forces combining to create the perfect storm will continue to collide and the storm itself will be a recurring event.

This apocalyptic vision has come more clearly into focus over the last two years. In fact, the occurrence and re-occurrence of Severe Acute Respiratory Syndrome (SARS) and highly pathogenic avian influenza (HPAI) have demonstrated that a devastating emerging disease may be closer to reality than to hyperbole.

Both national and international entities are struggling with the new phenomenon. What is urgently needed is a comprehensive approach, one that takes into account all the players in the drama, including but not limited to, ecosystem dynamics, biosecurity, public policy, animal medicine and husbandry, public health, and biomedical sciences. There are several national initiatives designed to facilitate these comprehensive planning and prevention processes. Such collaborations are also beginning to occur on a global scale as well. Despite the gloomy news played out over the occurrence of highly pathogenic avian influenza in Southeast Asia this past year, there was a definite bright spot as experts from the Office of International Epizootics, the Food and Agriculture Organization, and the World Health Organization, all came together, for the first time, to deliberate on how best to jointly work to prevent the continuing re-emergence of this dreaded disease. Similarly, it has been widely recognized that if we are to make any progress in preventing another global pandemic of SARS, it will be essential to thoroughly understand the

ecology of the disease agent and the trading practices and travel patterns that so facilitated its spread.

There is most definitely a blurring interface between human and animal health. Animals and people are inextricably interconnected. With global trade, habitat change, and a myriad of lifestyle issues surface and evolve, the connections are increasingly fluid, with lines constantly moving and shifting. The interface between human and animal health is becoming blurred to a point of indistinction. In fact, it is well established that 75% of all emerging diseases impacting people occur as a result of an animal pathogen moving into the human host, and so are classified as "zoonotic." The next step is to focus on the other interfaces, that is, the interface of disease agents with the environment, public policy, transportation issues, and technology.

Notes:

Avian Influenza: An Emerging Pathogen

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Avian influenza remains a growing problem for the poultry industry, and the number of highly pathogenic influenza outbreaks appears to be increasing. The virus can also be a zoonotic threat, which can complicate any control effort. To better understand the disease in poultry, the risk factors for disease outbreaks need to be understood before risk reduction measures can be instituted.

Avian influenza viruses are promiscuous viruses and can infect a wide variety of animal species, but the normal host range is primarily ducks, gulls, and other wild waterbirds [3,5]. The virus appears to be well adapted to these species, causing only an asymptomatic enteric infection with large amounts of virus being shed into the environment. For ducks, such as mallard or pintail ducks, the highest incidence of infection is usually in the fall, where you have large numbers of young naïve ducks congregating before flying south for the winter. The incidence of infection typically drops to low levels in the winter months, but virus can often be isolated all year long. The wild bird population therefore remains a reservoir source of infection that cannot be practically controlled [7].

Our common poultry species, including chickens and turkeys, are not natural hosts for avian influenza viruses [2,8]. If you experimentally infect chickens with wild duck isolates, typically the viruses will replicate at low levels, not be efficiently transmitted from bird to bird, and cause little to no disease [4]. In this situation, the virus will typically fail to maintain itself in the poultry population with or without human intervention. Therefore the flock's infection will not likely be recognized, except on routine serological surveillance. However, in part because of the segmented genome of avian influenza viruses, which helps to increase diversity in a viral population, and a high mutation rate, influenza viruses can rapidly adapt to a new host species. In some cases, a virus with the right "constellation" of genes can be introduced into poultry that can replicate and transmit well enough to establish an ongoing infection. Our modern poultry practices aid in the adaptation process for AI viruses because the high density of naïve birds increases the likelihood of virus transmission and opportunity for a virus to adapt for better replication and transmission in the new host. At some point the virus has changed (adapted) so much that it becomes a chicken or turkey virus and no longer replicates efficiently in the original host species, such as ducks [8]. Although, no specific genetic changes have been observed that can classify the virus as being a duck virus or a chicken virus, sequence data can provide an estimate of how long a virus has been circulating in the poultry population.

There are several recognized risk factors for the introduction of wild bird avian influenza viruses into our poultry populations. The first is direct access of poultry to wild birds. A good example of this is with turkeys in Minnesota that were raised on range in the 1980's and early 90's. Outbreaks of multiple subtypes of avian influenza occurred routinely in the fall, when you had a high incidence of infected ducks with the opportunity to commingle with turkeys. Once the virus was introduced onto a turkey farm, the virus could become adapted to turkeys and spread to other turkey farms by the

movement of infected birds and contaminated materials [1]. In the late 90's the practice of range rearing turkeys was greatly diminished in favor of confinement rearing. This management change reduced the exposure of turkeys to wild ducks, and the incidence of avian influenza outbreaks was greatly reduced.

A second source of exposure that has been implicated as a source of infection for poultry flocks is the bird's drinking water. For some poultry operations the bird's drinking water comes from surface sources, such as a lake, where wild birds often have free access. If the drinking water is not properly purified, avian influenza virus could be introduced by this source. The use of raw drinking water was suggested to be the source of AI outbreaks in Australia and Chile[9].

A third source of infection of influenza to turkeys is not from wild birds, but from pigs infected with swine influenza. Turkeys are susceptible to swine influenza viruses, and having a turkey farm and swine farm in close proximity is a risk factor for the introduction of swine influenza to turkeys. Infections with both classical H1N1 swine influenza and the more recent reassortant H1N2 swine influenza viruses have been reported [10,11].

A fourth risk factor for the introduction of avian influenza into poultry is the live bird marketing system, which is found in many countries around the world including the United States. Live bird markets offer a variety of birds that can be slaughtered and used for human consumption. Traditionally, this system was used as a way to maintain the freshness of the product where refrigeration was not required. However, in the United States, these markets cater to consumers who enjoy the variety of birds, including several types of chickens, quail, pheasant, ducks, geese and other birds, and the freshness offered by the markets. These markets are extremely popular with certain ethnic populations, and the consumer pays a premium price for the bird. This system however, provides an ideal environment to introduce and maintain avian influenza viruses into our poultry population. The domestic waterfowl, ducks primarily, are often raised on ponds where exposure to wild birds including ducks is common. This provides a high risk for domestic ducks to be infected with avian influenza. These infected ducks are often sold in the live bird marketing system, where there is close contact with chickens, quail, and other gallinaceous birds. These birds can become infected and will typically stay in the markets for a few days before being slaughtered and sold, which provides an opportunity for the virus to infect the naïve birds that are being introduced into the market periodically. Many live bird markets are never free of birds, and a continuous cycle of infection can be maintained, with the virus continuing to become better adapted to chickens. The virus in the live bird market system, although generally believed to be separate from our commercial poultry system, has been a nidus of infection for spread to our commercial poultry sector. One example is the H7N2 AI virus that has been circulating in the Northeast United States since 1994 and has been associated with at least 5 different outbreaks in industrialized poultry in seven different states[6]. The associated risk of waterfowl introducing avian influenza to chickens and other gallinaceous birds has prompted the Hong Kong government to prevent gallinaceous birds and waterbirds from being sold together. Additionally, they instituted periodic market closures to try and break the cycle of infection within the market. These changes have appeared effective at reducing the incidence of infected birds in the markets.

Currently, 15 different hemagglutinin subtypes of avian influenza have been described. However, surveillance studies in wild ducks show that H3, H4 and H6 subtypes are most commonly observed [7], but for poultry H5, H7 and H9 are more commonly observed. This apparent discrepancy might suggest wild ducks aren't the source of infection, but a more likely explanation is that some subtypes are more capable of establishing infections in poultry than others. Exposure to some subtypes may be higher, but few of these exposures lead to active infection. Unfortunately for the poultry industry, the H5 and H7 subtypes, although genetically extremely dissimilar, are the only subtypes associated with the highly pathogenic phenotype of avian influenza. Most H5 and H7 viruses isolated are low pathogenic, but some of these viruses can mutate to the highly pathogenic form. Some quickly, in just a few weeks, where others take months to years. The factors that lead a virus to become highly pathogenic are still not known.

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Notes:

What is New with Coronavirus?

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There are two themes to my paper. Firstly, that the host range of coronaviruses is greater than was generally thought, with potential consequences in terms of emerging diseases. Secondly, we have barely scratched the surface in respect of the discovery of coronaviruses in birds.

Host range of coronaviruses

We should not have been surprised that SARS coronavirus (SARS-CoV) was associated with several mammalian species – both wild and domestic – as well as in humans. The fastidiousness exhibited by many coronaviruses when it comes to growing them in the laboratory is not reflected *in vivo*. For example, the group 1 coronaviruses *Transmissible gastroenteritis virus* (TGEV), *Feline coronavirus* (FCoV) and *Canine enteric coronavirus* (CECoV) are not limited to their namesake host species; CECoV and FCoV can cause pathology in pigs[1,2]. Turkeys experimentally infected with *Bovine coronavirus* (BCoV) replicated the virus, resulting in pathology[3]. Saif and colleagues have shown that turkey coronavirus (TCoV) can replicate in the enteric tract of chickens, albeit asymptomatically[4].

Species within the genus *Coronavirus*

Coronaviruses have been placed in three groups, four if SARS-CoV is assigned to a separate group (Table 1). Generally, viruses in different groups have virion proteins with amino acid identities of <40%, whereas within a group identities are usually >60% (spike proteins sometimes being more variable). Perusal of Table 1 shows that we are of aware of several species of coronavirus in some hosts e.g. three CoV species in pigs; four in humans (including SARS-CoV and a very recently discovered species[5].

What it is to be a coronavirus

Coronaviruses are enveloped, approximately 120 nm in diameter, with large (20 nm), club-shaped surface projections (spike protein, S). Apart from the S protein, all CoVs have a smaller membrane glycoprotein (M), a few copies of the E envelope protein and a protein closely associated with the ~30 kb RNA genome (N). There are interactions between the S and M proteins, and between M and N proteins. The group 2 viruses have an additional structural protein, the haemagglutinin esterase protein (HE), which forms a layer of short surface projections.

Tropism of coronaviruses

Within a host species, a coronavirus replicates in many more tissues than the name of the virus would imply. This is well illustrated by avian *Infectious bronchitis virus* (IBV), which replicates at a myriad of epithelial surfaces – respiratory, enteric, kidney, oviduct[6]. A minority of strains of IBV are intrinsically nephropathogenic able to cause kidney-related mortality in experimentally infected chickens. Recently Yu *et al.*[7] have studied the pathogenesis of three isolates of IBV that caused proventriculitis. TGEV not only replicates at some enteric surfaces, as its name implies, but also in kidneys and lung. Some strains of TGEV are not enteropathogenic, likewise for BCoV.

Determinants of pathogenicity

There are doubtless many positions in the genomes of coronaviruses in which mutations can lead to changes in pathogenicity. For example, when we replaced the S protein gene of an attenuated strain of IBV with that from a pathogenic strain, it remained non-pathogenic, indicating that differences in genes other than S were responsible for the differences in pathogenicity (submitted for publication). Notwithstanding, its tropism in cell cultures had been changed[8]. The S protein determines the enteropathogenicity of TGEV and the nature of the neurovirulence of MHV. When the S gene of MHV was replaced with that of FCoV, the recombinant virus was then able to replicate in feline cells.

Thus (a) some hosts are known to be susceptible to two or more species of coronavirus; (b) that some species of coronavirus are to infect, indeed cause disease in, more than one species of host.

Coronaviruses in birds

Most of what we know about coronaviruses in birds is based on IBV[6], TCoV[9] and *Pheasant coronavirus* (PhCoV)[10] – i.e. limited to viruses of three economically important species. Surely there must be coronaviruses in other birds – and other coronaviruses that can infect our major domestic species?

The proteins of TCoV and PhCoV are no more different from those of IBV than the differences exhibited between serotypes of IBV. One might consider them to be host-range mutants of a single coronavirus species[11]. To date we consider TCoV to be enterotropic. Maybe there are some respiratory forms our there, as with TGEV and BCoV; we should keep an open mind. PhCoV is associated with respiratory disease in pheasants but also with nephritis

Are IBV, TCoV and PhCoV able to infect each other's namesake host? Experimental inoculation of TCoV into chickens resulted in replication in the alimentary tract, though asymptomatically4. A single experiment involving inoculation of chickens with a PhCoV resulted in no serological evidence of replication, though PhCoV does replicate well in embryonated chicken eggs[12]. Chickens, pheasants and turkeys are all in the order Galliformes. One would imagine that other species in this order are infected with group 3 coronaviruses, but what about other orders of bird? Might the latter be associated with coronaviruses yet to be discovered?

Might birds be susceptible to coronaviruses that we currently consider to be mammalian pathogens? When BCoV was experimentally inoculated into turkeys, it replicated, with pathology[3]. Therefore we have to keep an open mind to the possibility that turkeys might be infected, potentially with economic consequences, with coronavirus transmitted from domestic cattle – or from wild bovines. What viruses are present in wild turkeys?

Often overlooked is the discovery over 20 years ago of a coronavirus (puffinosis coronavirus) from the Manx shearwater (*Puffinus puffinus*[13]). It is a group 1 coronavirus, significantly different in sequence from the other known group 1 coronaviruses. Puffinosis involves vesicles on the feet, sometimes associated with conjunctivitis and paralysis of the legs[14]. Given that humans and pigs are each known to be infected with four and three species of coronavirus, respectively, might not poultry be susceptible to coronaviruses other than the known group 3 ones?

Recombination

Finally, a word about recombination amongst coronaviruses. There is no doubt that recombination is a feature of coronavirus replication; it has been shown experimentally, including for IBV, and with plenty of circumstantial evidence (sequence-based) for natural recombination of IBV in the field. Type II feline coronaviruses are most likely recombinants of canine and feline type I coronaviruses. However, we have little idea of the timescale of the recombination events. Much of what we see could have happened in 'ancestral' times. One would expect the generation of a successful recombinant to be a rare event. That said, there are some 40 000 000 000 chickens in the world each year; big numbers, offering opportunities for coronaviruses to jump in from other species, with the possibility of recombination.

We are aware of a dozen or so coronaviruses in <12 mammal species, making it likely that avian species are afflicted by more than just group 3 'IBV-like' coronaviruses. The only hard evidence for this assertion to date is puffinosis coronavirus, a group 2 coronavirus; this may be only the tip of an iceberg.

Acknowledgments

The author is supported by the Department for the Environment, Food and Rural Affairs, and the Biotechnology and Biological Research Council, UK.

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Table 1. *Coronavirus* species and groups

Host	Virus species	Acronym
	Group 1	
Swine	Porcine transmissible gastroenteritis virus	TGEV
Cat	Feline coronavirus	FCoV
Dog	Canine enteric coronavirus	CECoV
Swine	Porcine epidemic diarrhoea coronavirus	PEDV
Human	Human coronavirus 229E	HCoV-229E
Human	Human coronavirus NL63	HCoV-NL63
	Group 2	
Swine	Porcine haemagglutinating encephalomyelitis coronavirus	HEV
Cattle	Bovine coronavirus	BCoV
Dog	Canine respiratory coronavirus	CRCoV
Mouse	Murine hepatitis coronavirus	MHV
Rat	Rat coronavirus	RtCoV
Puffin	Puffinosis coronavirus	PuCoV
Human	Human coronavirus OC43	HCoV-OC43
	Group 3	
Chicken	Infectious bronchitis coronavirus	IBV
Turkey	Turkey coronavirus	TCoV
Pheasant	Pheasant coronavirus	PhCoV
	Group 4[b]	
Human, civet cat, raccoon dog; other?	SARS-coronavirus	SARS-CoV

Notes:

Emerging Diseases and Conditions in Layers

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The disease status of U.S. layers is quite stable, with very few significant new disease conditions to report. Some diseases that are present in layers in other parts of the world have fortunately not been introduced into the U.S. layer population, such as avian pneumovirus, egg drop syndrome, and very virulent IBD. Other diseases that have been present in the U.S. layer population have either been eradicated (exotic Newcastle, avian influenza) or are being effectively controlled by biosecurity management and vaccination (*S. enteritidis*, Marek's disease, infectious laryngotracheitis, and fowl pox).

Normal layer mortality is in the range of 0.08-0.15% per week. This will vary by strain and age of the flock. Any of a number of diseases can cause temporary increases in mortality, but the major causes of "normal" layer mortality are *E. coli* peritonitis, soft bone conditions, prolapse/peckout, and gout. A review of the routine disease conditions of layers is provided at the annual meeting of the U.S. Animal Health Association (1).

E. coli Peritonitis

One of the most challenging causes of mortality in adult layer flocks is *E. coli* peritonitis. Birds die quite suddenly, usually in good body condition and a high rate of egg production. Lesions consist of a serous to caseous yolk-colored exudate in the abdominal cavity. *E. coli* is most frequently isolated, although a number of other bacterial types can be found, including *Salmonella*, *Pasteurella*, *Staphylococcus*, *Streptococcus*, and *Enterococcus*.

There are two separate time periods when layers are more susceptible to peritonitis for different reasons. Early peritonitis occurs around the peaking period of 23-32 weeks of age and is generally seen in multi-age complexes. This seems to be of respiratory origin and is often associated with other primary respiratory diseases, such as bronchitis and mycoplasma (MG or MS). Poor air quality, with regard to dust and ammonia levels, definitely exacerbates the problem. To date, there is no evidence that pneumovirus or *Ornithobacterium rhinotracheale* are primary pathogens leading to peritonitis in layers in the U.S. Occasionally, no predisposing factors can be identified and it seems the *E. coli* is very virulent on its own and is acting as a primary disease agent, much like fowl cholera.

Late peritonitis cases occur after about 50 weeks of age and seem to be more related to vent trauma and ascending infections from the cloaca. This can result from sub-lethal vent cannibalism or sub-lethal partial prolapse, and is exacerbated by excessively large egg size, fat accumulation in the abdomen, and high light intensity.

Treatment or prevention of the early type of peritonitis is more successful than for the late type. Antibiotics approved for adult layers are very limited and are often relatively ineffective in peritonitis control. Other treatments methods have been attempted with little success, including probiotics, formaldehyde/organic acid feed additives, and orally-administered *E. coli* antibody.

Prevention may best be accomplished through better control of the inciting primary respiratory diseases. This may include a more broad-spectrum bronchitis vaccination or better mycoplasma vaccination. MG control with vaccination is sometimes difficult to accomplish, or may not persist for the entire production cycle. MS has historically been felt to have little effect in layers, but field evidence suggests it can be a factor in initiating peritonitis. This suggests the industry could use a modified live MS vaccine. Purposely exposing growing pullets to the wild type of MS may remove that stress from the early lay period and reduce the tendency of young layers to develop peritonitis.

In recent years, the layer industry has increasingly adopted the use of autogenous *E. coli* bacterins for early peritonitis control. This has met with quite good success. Autogenous products are difficult for layer producers to deal with from a regulatory viewpoint, so a generally licensed *E. coli* bacterin would be useful for the industry.

Osteomalacia / Osteoporosis

Layers are susceptible to a couple bone conditions that may be related and often confused. Early in the production cycle, layers may not be consuming sufficient calcium or phosphorus to supply their daily needs for eggshell formation and bone regeneration. The result is a soft bone condition, osteomalacia, characterized by very pliable bones, crooked keels, collapsed ribs, birds going down in the cage, reduced production, and increased mortality. It seems difficult to distinguish between calcium, phosphorus, and vitamin D_3 deficiency at this stage. Close attention to daily intake of these nutrients should prevent development of osteomalacia. Of the two conditions, osteomalacia is probably the more significant production problem. Early problems with osteomalacia make cause a flock to be more susceptible to osteoporosis later in life.

Osteoporosis is the more commonly publicized condition. It is related to advancing age of the laying hen and is characterized by thin, brittle bones. Typically, fractures of long bones occur when handling the birds, resulting in welfare concerns and bone fragments in spent hen meat. Osteoporosis seems to be difficult to prevent with nutritional or management alteration.

Gout or Urolithiasis

Layers seem to be susceptible to kidney damage that results in mortality with lesions of gout or urolithiasis. Young growing pullets are felt to be more susceptible to kidney damage from various causes, but the mortality from kidney failure may not occur until the flock is mature and consuming higher-calcium layer feed. Kidney damage could result from nephropathogenic strains of infectious bronchitis virus, certain mycotoxins, nephrotoxic drugs, or water deficiency. However, in most cases, abnormal intakes of

calcium or phosphorus are felt to be the cause. Excess intake of calcium in young growing pullets will damage the kidney. This could occur from accidental delivery of layer feed to growing pullets, use of pre-lay feed too early, or use of particle-size calcium in growing feeds, allowing individual birds to selectively consume too much calcium. Phosphorus has been shown to exert a protective effect against calcium damage, and field experience has shown that grow feeds with relatively low available phosphorus tend to relate to more gout mortality later in production.

Kidney damage cannot be reversed, but mortality can usually be reduced by acidifying the diet with ammonium sulfate or ammonium chloride at gradually increasing levels up to 1.0% of the diet (2).

Focal Duodenal Necrosis (FDN)

In Pennsylvania in 1996, an enteric condition was found in layers that seemed to be associated with some performance problems. The only notable gross lesion was ulcers and/or raised gray/green patches in the duodenum, and rarely in the proximal jejunum. Microscopically, there are ulcers on the tips of villi, populated with large Gram-negative rods. Underlying gut lining is filled with heterophils. The only significant clinical effect is a reduction in egg weight for several weeks compared to normal. Egg weight may be slow to increase early in production, or may temporarily fall later in production. The rate of egg production is generally normal, but rarely may show a slight decline. Affected birds may appear to have slightly pale combs, but droppings are normal and mortality is not elevated. All strains of layers seem to be equally susceptible to FDN and it most often is found in multi-age layer complexes. A spent hen project in Pennsylvania in 2000-2001 examined 60-65 hens from each of 46 flocks, representing seven companies. 39% of the flocks had lesions of FDN, and 1-6 birds were affected per flock (up to about 10% incidence).

A number of bacterial types have been isolated from affected gut tissue, but there is no proven cause of the disease. No viruses or spirochetes have been isolated. There is no known association with mycotoxins, biogenic amines, enteric parasites, or feed ingredients. The condition does respond to antibiotics, such as tetracyclines, neomycin, bacitracin, tylosin, and erythromycin. Recurrence after treatment is possible. Probiotics are helpful, but organic acids are not effective.

Although seemingly minor compared to most other diseases, FDN is moderately significant to layer producers. It reduces egg income due to lower prices received for the smaller size eggs produced. This, in addition to the antibiotics that may be administered, cost a producer an estimated $12,000-18,000 per 100,000 layers.

FDN does appear to be spreading geographically. After first being discovered in Pennsylvania, it has now been recognized in at least six other states, mostly in the Northeast. It may be more common than realized, as it is only diagnosed by necropsy of average-appearing layers, and grossly inspecting the duodenum for typical lesions.

There are no published reports of this condition in refereed journals yet, but many should be acknowledged who have been involved in investigating FDN in Pennsylvania and generating this information, including Sherrill Davison, Patty Dunn, Bob Eckroade, Eric Gingerich, David Kradel, Robert Norton, Dan Shaw, Eva Wallner-Pendleton, Andre Ziegler, and likely others.

Peripheral Neuropathy (PN)

In recent years, a neurological disease has been recognized in layer pullets that closely resembles Marek's disease, but is not caused by the Marek's disease virus. The syndrome has been termed "peripheral neuropathy" and the cause is not known at this time (3).

The clinical symptoms associated with peripheral neuropathy (PN) are paralysis of the leg muscles and birds unable to stand, as seen with nerve involvement in Marek's disease (MD). Pullets in the age range of 6 to 16 weeks are most often affected. Mortality is elevated in typical commercial conditions and can reach 2-5% of the flock. Postmortem lesions consist only of slightly enlarged sciatic nerves in the legs of some affected pullets. Grossly visible nerve enlargement may be seen in only 15-25% of affected pullets. On microscopic exam, the nerve lesions may not be distinguishable between PN and MD, as both involve the milder B-type histologic lesion. The best way to differentiate the two is that MD should also produce gross and microscopic tumor lesions in other internal organs, such as the liver, spleen, kidneys, proventriculus, brain and ovary. PN will not cause internal organ tumors, but rather just the relatively minor peripheral nerve lesions.

Experimental evidence suggests this syndrome may be an autoimmune reaction against nerve tissue, triggered by the bird's immune reaction to other common vaccinations. It also appears there is a genetic aspect, as it is recognized only in certain strains of layers and is more prevalent in birds with certain blood types within those strains.

Marek's disease vaccination neither prevents this disease nor appears to cause it. At this time, there is no known method to prevent or treat the condition. Layer strains known to exhibit this condition should be expected to have some mortality from PN in the growing period.

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Notes:

Emerging Diseases and Conditions in Broilers and Broiler Breeders

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Excellent reviews of current and emerging conditions in broilers and broiler breeders are prepared every year and presented at the annual meeting of the United States Animal Health Association (Smith, 2003, Stewart-Brown, 2002). The top 10 diseases/conditions concerning broiler production veterinarians in 2002-2003 are presented in Table 1. While the rankings on the list may vary slightly from year to year depending on current disease challenges, the list itself remains relatively consistent.

Even though the diseases that challenge the animals under the care of US broiler veterinarians have changed very little over the last few years, the challenges facing these veterinarians have become very different.

In order to gauge how the challenges facing broiler production veterinarians have changed, a questionnaire was prepared and sent to a representative sample of these professionals. The survey was designed as a series of questions to which the participants responded by circling a number from 1 to 5 with a response of 1 meaning that they were in agreement with the statement and 5 meaning that they disagreed with the statement. While this survey was admittedly non-scientific, the consensus of opinion from veterinarians in widely different geographic regions was surprising.

Four of the ten questions elicited virtually unanimous agreement in their responses. Almost all respondents were in strong agreement with the first statement on the survey: Issues other than bird health continue to occupy more and more of my time. Factors cited that are diluting time spent on bird health issues include animal welfare, antibiotic usage, and trade issues.

Another question: I am confident that the U.S. poultry industry is ready in the event of a bioterrorist attack, elicited a very high level of disagreement with the statement. In response to a related question: Recent challenges from Avian Influenza have caused me to revise my company's biosecurity program, participants indicated that they all had improved biosecurity yet virtually all felt that a bioterrorist attack on the poultry industry would be a disaster. One interpretation of this data is that while veterinarians in broiler production feel that they have made improvements in the biosecurity of their respective companies, they are still not confident that local, state, and national programs of disease control will be adequate if faced with a very virulent and highly contagious challenge.

Another virtually unanimous response was recorded in disagreement with the statement: Campylobacter is a disease that I understand and can control. Issues of food safety occupy a great deal of the time of veterinarians in broiler production and the poultry genetics industry and processing plant results show that progress is being made in the area of Salmonella control. Campylobacter, however, is an organism that is much discussed yet few tools are currently available to understand and control it.

The last virtually unanimous response in disagreement was recorded to the question: Unavailability of antibiotics will <u>not</u> seriously impact my ability to practice

poultry veterinary medicine. There appears to be a misconception that broiler veterinarians misuse antibiotics and are not to be trusted to use these tools in a professional and responsible manner. This is categorically untrue. There is not a broiler veterinarian in the field that does not always consider the cost to benefit ratio, residue avoidance, and resistance issues when prescribing antibiotics for a flock of sick birds. The loss of these tools either through legislation or public misconception represents a serious threat to grower relations, animal welfare, and disease control. Emotions and/or misinformation must not rule. Decisions regarding the availability and use of antibiotics to treat the animals for which veterinarians in the broiler and poultry genetics industries are responsible must be based on sound carefully reviewed science.

Questions asked regarding individual diseases elicited little consensus in the responses. Most respondents felt that spiking mortality syndrome was around but was being managed. Ornithobacterium rhinotrachealae and chicken anemia virus were both considered to be agents capable of causing clinical problems but were at this time not causing any significant problems. Respondents in the Southeast reported issues emerging regarding Newcastle disease. These issues may be due more to viral interactions in vaccinated flocks rather than change in pathogenicity of the Newcastle virus. Other challenges that were mentioned as being seen more frequently than in the past include Clostridial diseases and histomoniasis.

Two questions at the end of the survey asked respondents what emerging disease/condition will be most or least important in the future. In reviewing the answers to these questions, a new disease, termed "political disease", was referenced repeatedly as the most important emerging disease. We live in the information age and even before the severity and extent of an outbreak is assessed, people around the world are reading about it via the Internet and trade embargos are being put in place. Isn't it interesting how quickly these barriers are put up and how slowly they are removed? The emergence of these "political diseases" has seriously complicated the lives of veterinarians in both broiler production and the poultry genetics industry and the problem seems to get worse and worse each year.

Several respondents were also concerned about a condition that has been around for a long time but could be classified as emerging because it is increasing in frequency and severity. This condition is known as Management Deficiency Syndrome or MDS. Clinical signs include watery eyes of people entering the chicken house, birds inadvertently without feed and/or water for varying periods of time, and a myriad of other problems. As farms have gotten larger and buildings have gotten more automated, this problem rather than getting better, as one might guess it would, has gotten worse. Successful husbandry requires thorough knowledge of both the species we are raising and the mechanical systems involved. Reducing the prevalence of this problem will require a significant investment of time in training and continuing education for both growers and service people. All involved in animal agriculture need to constantly remind themselves that least cost may not always be the best for the birds or the most profitable avenue and a machine will never be as good at telling us what is needed as the chickens themselves.

The last two questions asked what academia and regulatory officials could do to help with emerging diseases/conditions. For each the answer was clear. To people in academia the message was don't assume what we need: ask us and work with us. Veterinarians in broiler production and poultry genetics both admit that more and more of

our daily lives are occupied with things such as "political diseases". In the meantime variant viruses are still emerging and conditions that don't offer an apparent solution are happening. We need help and support from academia to delve more deeply into these problems and help us understand and deal with them. We also need help developing and administering training programs and continuing education programs for growers and service people to combat MDS.

To the regulatory officials the message is help us, work with us, don't complicate our lives. We need help with these "political diseases". Import/export requirements are supposed to be treaties negotiated between countries not rules imposed based on the most current diagnosis posted on the Internet. Try to understand our business and the challenges and economic pressures that we face. In the experience of this author, many of our trading partners and some of our own regulatory officials do not know what the NPIP is or the testing required to participate in this program. It might be argued that the NPIP needs to be changed in some way, but the fact is that the organization has a long and illustrious history. We should capitalize on this and use it to establish confidence in our poultry health surveillance programs and reduce the possibility of embargos due to "political diseases".

One of the respondents added a final question to the survey: What can industry do to help with emerging diseases/conditions? His answer was the same for each of the last three questions and provides a good foundation for us all. The answer was: listen to each other and work together. Too often we all get caught up in whatever is the daily crisis and lose sight of the big picture. Our mission as poultry veterinarians in industry, academia, and government should be to provide the best care to the animals we are responsible for and to protect the health of our domestic and international customers.

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Table 1: Top Ten Broiler/Broiler Breeder Disease Issues in 2002 – 2003

Disease	Responses
Infectious Bronchitis Virus	8
Gangrenous Dermatitis	4
Necrotic Enteritis	4
Coccidiosis	4
E. coli	4
Mycoplasma	4
Fowl cholera	3
Infectious Laryngotracheitis	3
Sudden Death Syndrome	3
Leg Problems	2

(adapted from Smith, 2003)

Notes:

Emerging Diseases and Conditions in Turkeys

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In preparation for this report several US industry professionals and veterinarians involved in turkey production, were contacted to inquire about the health issues. The turkey industry reports several disease challenges varying by geographical regions within a state and across the United States. This report will list, in alphabetical order, the challenges by disease.

1994 – 2004

New, Emerging Diseases & Condition

1. ORT
2. APV
3. PEMS
4. Cellulitis in turkeys
5. Cochlosoma infection (protozoal enteritis)
6. Avulsion of the common retinaculum, lateral intertarsal tendon (Julian, 1984; Crespo, 2003)

Re-Emerging Diseases & Conditions

1. Blackhead
2. Necrotic enteritis
3. Exotic or Velogenic Newcastle (as a threat to poultry health and exports)
4. Avian Influenza
    a. High path and low path H5, H7 and related international trade barriers
    b. Other low path AI strains (hindering trades, depopulation of primary breeds)
5. Erysipelas
6. MG
7. Breast blisters (developmental)
8. Leg issues (developmental)
9. Fractures

Sporadic

1. MS
2. Bordetella avium
3. Colibacillosis (HE and/or Coccidiosis combination)

Poult (viral) enteritis was a cause of relatively higher early morbidity and mortality, especially in the lower Midwest and Southeast. Astrovirus was identified by PCR and enterovirus was identified by virus isolation. Respiratory problems with AMPV-1, E. coli, ORT and BART are problems in some flocks, resulting into poor performance and excessive mortality. ORT is diagnosed as a part of the problem with no commercial vaccine available. Fowl Cholera has been diagnosed more frequently in the Southeast associated with the wetter season and was particularly severe in some breeder operations. Osteomyelitis (OM) continues to be a problem in some flocks. Other diagnoses of particular interest include, Blackhead, Cellulitis and APV.

Turkey **production** totaled 5.65 billion pounds in 2003. Production declined 1.25% (71 million pounds) for the year 2003, only the third annual decline since 1982 (Sparks Companies Inc, March 2004. Heads slaughtered was down 1.3% and a 0.2 pound (0.09%) increase in average live weight. Declines were mainly the result of poor profitability in predominately further processed items from heavy toms. Ready-to-cook production in 2004 is expected to be 2-3% lower than 2003. Overall domestic demand for turkey

products is strong, while export bans have been limited to due chicken outbreaks of high path and low path AI. Exports in 2004 are expected to reach 514 million pounds. Export bans and higher feed costs are expected to be the two major challenges for 2004.

Over the past decade, the industry has adapted its **production systems** from multi-age facilities to single-age operations. An informal survey (Clark, 2001) was conducted of the US Turkey Industry to identify single-age production systems (all-in/all-out, brood-n-move); in 2001 there was 26% single-age production, compared to 1995 the estimate was 19%. This trend continues. The increase in single-age production is due primarily in an attempt to control/minimize disease challenges specific to different areas.

The lack of effective **therapeutic agents** remains to be a concern of the industry, including the loss and potential loss of efficacious treatments for bacterial diseases. The judicious use of antibiotics, including fluoroquinolone, appears to be reducing mortality in many turkey flocks. The turkey industry wants to ensure that any CVM antibiotic resistance policy is scientific and results in no loss of available drugs unless there are clear scientific evidence those drugs pose a danger to human or animal health.

Avian Influenza has been diagnosed in various chicken broiler cases but outbreaks in turkeys have been limited. Broiler AI cases have negatively affected turkey exports. The US remains free of High Path AI. Highly pathogenic avian influenza was not detected in the United States. A large outbreak of Low Path AI (Orthomyxovirus) was diagnosed in Virginia turkeys in 2001, resulting in large-scale depopulation and ultimate eradication of the disease. OIE is considering defining notifiable avian influenza (NAI) as an infection of poultry caused by any influenza A virus of the H5 or H7 subtypes or by any AI virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as an alternative at least 75% mortality); NAI viruses can be divided into highly pathogenic notifiable avian influenza (HPNAI) and low pathogenicity notifiable avian influenza (LPNAI).

Avian Metapneumovirus (Avian Pneumovirus; APV) Infection in turkeys causes respiratory disease of all ages. Avian Metapneumovirus in the US is distinct from TRT virus in other countries. It is limited to the upper Midwestern states and is a common cause of secondary colibacillosis. In 2003 the incidence is reported to be slightly higher than the previous year.

Blackhead: The sporadic incidence of histomoniasis in turkeys was about the same across the US in 2003, than in 2002. In the Southeast and West, particular locations reported Blackhead both in commercials and breeders. Control of this disease is impaired by not having available an effective, approved treatment. New research and field observations support the opinion that direct transmission is possible where the *Histomonas* are able to infect turkeys for a short period after being excreted. Since oral transmission seems impossible, it is believed that the mechanism for this is "cloacal drinking" (McDougald, 2003); McDougald suggests that the *Histomonas* protozoa in the feces, directly infects new turkeys via the cloacal route. Blackhead can occur in most weather conditions but is a greater problem in warmer months. Occurrences of the

disease in turkey flocks have sometimes been associated with the recent cleanout of a nearby broiler breeder farm that was asymptomatically carriers of the disease.

Bordetella avium (Coryza), caused by *Bordetella avium*, is known by many names, including BART, Bordetella, ART, Snick, etc. Turkeys between 2 - 8 weeks of age are most severely affected, though any age bird is susceptible. Bordetella continued to be a sporadic problem and cause of respiratory disease and subsequent immunosuppression on poorly managed farms. *Bordetella avium* continued to be a nagging cause of respiratory disease, depressed weight gain and secondary colibacillosis.

Breast Blisters and Breast Buttons: A breast **blister** is a dilation of the sternal bursa with or without fluid and/or cords. A breast **button** is a focal ulcerative dermatitis (FUD) usually located in an unfeathered area of skin over the keel. Focal ulcerative dermatitis (FUD) has been described as a chronic active ulcer with raised edges that occurs on the unfeathered portions of the breast skin on or near the midline over the anterior keel of turkeys, primarily in toms. An increased incidence of FUD has been associated with increasing age, locomotor difficulty, litter moisture, and litter ammonia. Breast **blisters** and breast **buttons** are noted to be more prevalent in birds with poor breast feathering (Miner *et al.*, 1975b; Helms *et al.*, 1996). **Blisters** and **buttons** do not necessarily occur together. Breast lesions must be trimmed at processing, resulting in a downgrading of the carcass and economic losses (Gonder *et al.*, 1987). Over the past 15 years, the incidence of breast **buttons/blisters** and growth rate (i.e., ADG) has both increased. **Blisters** are more common in the summer months and associated with poor feathering over the breast. There is an association between ADG and the incidence of breast **blisters**; such as flocks with high ADG have a higher incidence of **blisters**. Poor quality, coarse litter ("sticks") is associated with **buttons**. Breast **buttons** are not caused by pressure on the skin. To control breast defects, proper litter management is critical, including using fine, soft shavings and keeping it dry.

Breeders: Two conditions have been noted in breeders, especially in hens (1) swollen feet caused by *Staphylococcus aureus* and (2) conjunctivitis and blepharitis (Aziz, 2004) caused by *Staph. aureus* and *E. coli*.

Cannibalism is often associated with an aggressive flock, particularly in the spring season as day-length increases. Some report a breed-association with aggressive behavior.

Cellulitis: *Clostridium septicum, C. sordellii, C. colinum, C. perfringens, or Staph. aureus can cause cellulitis. E. coli* and *Strep.* have occasionally been isolated from birds diagnosed with cellulits. Cellulitis in turkeys appears as excess mortality in older birds, around 16 - 18 weeks of age. It has been reported as early 7 weeks of age. Some cases present with dead birds having "bubbly tail", fluid filled blisters associated with broken feather follicles around base of the tail. Other cases will have dead birds with a gelatinous accumulation of fluid under the skin, usually along the thighs and breast. The dead birds decompose very quickly. Culturing the organism is difficult. In the Midwest cellulitis of the tail and lower abdomen continued to be a sporadic occurrence on a few farms.

Cholera (Pasteurella multocida) infections were reported as problems in the Southeast, lower Midwest and upper Midwest. A lower incidence of Cholera occurred compared to previous years, and the severity of the disease was muted. Cutaneous manifestations were interestingly common this year. It was a sporadic problem on a limited number of farms. Fowl cholera was identified is a few flocks, primarily heavy toms approaching market age.

Coccidiosis is a disease that is caused by the *Eimeria* protozoan parasites that develop within the intestine. The efficacy of currently used approved anticoccidial medications and vaccines has controlled, to a large degree, severe clinical coccidiosis in the field. Subclinical disease and the presence of coccidia oocysts are commonly diagnosed.

Colibacillosis, caused by *E. coli*, continues to be a cause of mortality in turkeys. The only approved, efficacious product for the control of mortality associated with *Escherichia coli* is enrofloxacin, a fluoroquinolone.

Erysipelas continues to be a sporadic diagnosis.

Fractures are reported particularly in heavy toms, prior to slaughter. Fractures can lacerate the femoral artery resulting in acute death. Many fractures appear after toms make a sudden jump. Factors such as selection for heavy body weight, lack of exercise, angular bone deformities and handling might contribute to (stress, spiral) fractures (Crespo, 2002); fractures have been associated to heavy body weight and not insufficient bone structure (strength). One colleague observes mostly valgus deformities of the femur, which might put undue stress on the bone leading to spiral fractures. Rapid growth rate and sexual maturity make toms excitable and jumpy (Ghazikhanian, personal communication, 2004); the nature of femur bone (having spiral growth pattern and an angle against the body, in comparison with tibia bone) makes this long bone susceptible to overt fractures or hairline fractures upon stress (jumping). Hairline fractures become compound fractures once other heavy and aggressive males victimize (cannibalize) the "downer" and "injured" subjects. To resolve issues with leg fractures some consider varying lighting programs and/or reducing light intensity; it is observed that if it is extremely bright in the finisher barns, the birds are more active (and heavy) and therefore at risk to fracture. Managing light intensity and lighting programs is difficult in most US facilities because most barns are curtain-sided, which allows the natural sunlight to light the barn. Another area to investigate is any handling stresses, such as moving to finisher barns at 4 – 8 weeks of age, which might contribute to microfractures.

Heat stress and associated mortality has been a sporadic problem.

Late Mortality may be defined as mortality, in excess of 1.5% per week, in toms 17-weeks and older; mortality is not diagnosed to a specific disease or cause. Excess cumulative mortality of 5 – 10% in toms prior to slaughter has been reported. One suggestion for some late mortality (Ghazikhanian, personal communication, 2004) is that early rapid growth in heavy toms puts too much pressure on cardiopulmonary organs (supply organs) to meet the requirements (oxygen and nutrients) for demand organs

(massive breast muscles) to achieve its genetic potential; thus the cardiopulmonary systems becomes easily exhausted as body weight increases, in certain males, especially under higher density (pen-mate tension). Such inefficiency would cause heavy toms to sit (rest) alongside of the walls where they feel secure, weak and timid birds would invite aggressive toms. Cannibalism or hypertension due to stress hormones would cause mortality. Tired and exhausted sitting toms do not necessarily have clinical bad legs. A recent survey of late mortality in toms at US turkey complex with predominately one breed, showed 12% cardiac-related mortality (perirenal hemorrhage, aortic rupture, etc), 36% fractures (femur, tibia), 52% TDC, 12% OM, 56% pododermatitis.

Leg Problems may be defined as lameness, particularly in toms, several weeks prior to slaughter. Leg problems are attributed to various conditions, including, pododermatitis, fractured femurs, fractured tibia, osteomyelitis (OM), tibial dyschondroplasia (TDC), spondylolisthesis, Shaky Leg, etc. Leg problems may be classified as either an abnormality or leg weakness (Ghazikhanian, personal communication, 2004). A **leg abnormality** refers to phenotypic deformities caused by developmental (i.e., valgus, varus) or infectious process (i.e., synovitis, osteomyelitis). On the other hand, **leg weakness** can be physiological (cardiopulmonary exhaustion), structural (changes in tendon and ligament attachments, or joint capsules), and/or chemical (enzymes).

Mycoplasma gallisepticum (MG) in turkeys can cause a severe respiratory disease and subsequent airsacculitis condemnations at processing. The primary breeders have remained free of MG. A few sporadic cases of MG were reported in 2001. In 1999 - 2000 a MG outbreak in chickens and turkeys in North Carolina was eradicated. One small, limited outbreak of MG was diagnosed in the Southeast.

Mycoplasma meleagridis (MM) continues to be a sporadic diagnosis.

Mycoplasma synoviae (MS) infections (infectious synovitis) is one cause of synovitis. It may be present in flocks 10-12 weeks of age with typically low mortality and low morbidity. MS was sporadically reported this year. The primary breeders have remained free of MS.

Newcastle Disease Virus (NDV): Avian Paramyxovirus Serotype 1 (APMV-1) is the causative agent for both the endemic, mild, even asymptomatic, respiratory disease found in both turkeys and chickens and the diseases of higher significance previously called mesogenic or velogenic Newcastle Disease. For purposes of trade, control measures and policies, the OIE chapter (Ch 2.1.15, OIE Manual of standards Diagnostic Tests and Vaccines 2000) on Newcastle Disease Virus (NDV) defines Newcastle Disease as an infection of birds caused by a virus of avian paramyxovirus serotype 1 (APMV-1) that meets one of the following criteria for virulence utilizing an intracerebral pathogenicity index (ICPI) in day-old chicks or a characteristic pattern of amino acid residues. OIE has removed any reference to the NDV pathotypes and only uses ICPI and genetic sequencing as the criteria for reporting Newcastle Disease. Exotic-type Newcastle disease was not detected in turkeys nor was it detected in any poultry outside of the 2003 quarantined areas in the Western US. It is noteworthy that one localized diagnosis of

Exotic-type (made in backyard chickens) was hundreds of miles from any significant commercial turkey operations. Although exotic NDV has not been diagnosed specifically in turkeys, it did have a negative affect on turkey exports from affected states and international trade.

Necrotic enteritis has been diagnosed in the US. Especially in Europe, necrotic enteritis is a problem in part due to a lack of effective control, following the ban on gut health promoting medicines. Necrotic enteritis is caused by Clostridium perfringens, type A or C. The severity increases with increases in a-toxin formation, formation varies with strains. Clostridium perfringens is normally and commonly found in the environment and intestines. In turkeys, Droual (1994, 1995) reports that necrotic enteritis is more common in males (85%), from 6-11 weeks of age, with concurrent disease (46%), such as Coccidiosis (31%), Hemorrhagic enteritis (15%), Ascaridiasis (worms) (7%). Clinical signs (Droual; Gazdzinski, 1992) include sudden increase in mortality, depressed birds and decreased body weight (growth suppression). Upon necropsy, there is dehydration, crop mold, dilated small intestine with white foci visible and thick diphtheritic white-brown membrane. White cecal cores are occasional.

Ornithobacterium rhinotracheale (ORT) has been diagnosed throughout the US. Management systems, such as brood-and-move have increased the exposure of ORT-naive birds to ORT in the finisher barns, resulting in respiratory disease and mortality in some operations. Most recently ORT was a problem in a limited area in Upper Midwest commercial flocks.

Osteomyelitis (OM) is defined as inflammation of bone, commonly as focal necrosis under the epiphyseal cartilage (growth plate) of long bones. *Staph. aureus*, *E. coli* and *Actinomyces* are common bacterial isolates. OM is commonly found in the femur (thigh bone), tibiatarsii (drumstick-bone) and occasionally the vertebrae (spine). OM is more common in toms than hens, from 12-21 weeks of age.

PEMS (Poult Enteritis Mortality Syndrome) is defined as an infectious, transmissible disease of uncertain, but probable viral etiology, which typically affect young turkeys between 7-28 days of age. PEMS is characterized by diarrhea, dehydration, weight-loss, anorexia, immunosuppression, growth depression (>40%), and mortality (>2% between 7 and 28 days). Two clinical forms of PEMS have been recognized; the most severe is called Spiking Mortality of Turkeys (SMT) while the milder form has been named Excess Mortality of Turkeys (EMT). Turkey Coronavirus (TCV) has been associated with some of the PEMS cases. The Southeastern US turkey industry is continuing to be plagued by PEMS, even with good control of coronavirus (TCV).

Poult Enteritis of unknown etiologies has been less of a problem this past year. Some cases of enteritis are diagnosed as TCV and others progress to be identified as PEMS (mortality). But many cases are still not diagnosed with a specific cause, although viral etiologies are commonly suspected. It is typically observed between 2 - 5 weeks of age. Some areas have associated enterovirus, rotavirus and/or astrovirus, sometimes complicated by enteric flagellate protozoa, with their poult enteritis cases. In the

Southeast viral enteritis is still a problem in young poults and associated mortality in some cases reaching the level compatible with a diagnosis of PEMS. Overall enteritis was much improved throughout the US compared to the previous year.

Protozoal Enteritis attributed to flagellated protozoa (*Cochlosoma, Trichomonas* and *Hexamita*) are common in the summer months throughout the Southeast and Midwest. Protozoa severely complicate TCV, PEMS and other enteric diseases. Since about 1995, *Cochlosoma* has been diagnosed in several states, including North Carolina, South Carolina, Virginia, West Virginia, Missouri, Arkansas and Texas. In the southeastern United States, some veterinarians have associated *Cochlosoma* as a primary cause of enteritis. Boucher reported (1999; 2000) that the organism is very resilient. In their laboratory, it remained viable from pH 3 – 12 and also remained viable with cryopreservant for at least 3 months. Drying (at 60°F and 99°F for 24 hours) destroyed the organism. Formalin, phenol and quaternary ammonium will effectively kill *Cochlosoma*. However, it did survive exposure to 5.25% sodium hypochlorite (bleach) and iodophor disinfectant (iodine). Boucher (1999; 2000) studied the adverse effects *Cochlosoma* with enterovirus has on performance; their study showed a 24.4% decrease in feed intake and a 37.3% lower weight gain, resulting in an 18% higher feed conversion, compared to controls. Turkeys infected with *Cochlosoma alone* had a 12.5% increase in feed consumption and a 17.1% increase in feed conversion in comparison to negative controls (Bermudez, 2002).

Round Worms (Ascaridia dissimilis) infestations are common. In July 2000 Fenbendazole was approved for the removal and control of gastrointestinal worms. It is the first and only product approved as a medicated feed additive for use in turkeys.

Salmonella has been a problem for some producers. It has been associated with poor poult quality issues, resulting in excessive poult mortality. Sporadic diagnosis of this disease has been made this past year.

Shaky Leg Syndrome appears to be related to the extremely rapid growth in the birds. Some colleagues associate the syndrome with TDC, especially if the cartilage plug is located extremely medially providing minimal bone support. One commented that it might be associated with the calcification of the leg tendons; genetic selection for rapid growth rate and large breast conformation (genetics) has been associated with shaky legs. This is related to rapid early growth, early age to sexual maturity, early closure of epiphyseal (growth) plates of long bones and conformation affects balance and posture. Most rapid skeletal growth occurs between 2-8 weeks of age. At about 12 weeks old, 95% long bone growth is complete and only 45% muscle mass [as reported in the 1980's]. This rapid early growth might lead to early minor deformities and stress on bones, which later manifest as major lameness when the remainder of muscle mass is accumulated. Occurrence and severity vary depending on geographic areas, bird weights, etc. Some breed specific associations have been noted. Also consider that increased production/growth, leads to increase feed and water consumption, which results in more feces and decrease litter quality and resulting increased leg problems. Bird density and/or poor litter conditions could cause stress, resulting in shakiness of the legs or part of the

body of such toms. In many instances shakiness is a transient syndrome in 12 to 14 week old flocks and could disappear at 17 to18 weeks of age.

Spondylolisthesis (Kinky-Back) is not commonly reported. It is a deformity (kyphosis) of the spine (thoracic vertebraeT_{5-7}) causing pressure to put on the spinal cord because of kinky-back causes a partial paralysis of the legs. It is more common in males than females, usually occurring after 10 weeks age. Several birds in flock may be affected presenting with a shortened appearance, arched back and may sit on their hocks (with feet raised). The neck is extended and the birds may be "backpedaling". There is actually a rotation of one vertebra, which compresses the spinal cord, causing paraplegia. The cause is unknown.

Tibial Dyschondroplasia (TDC, Osteochondrosis) affects the cartilaginous growth plate, resulting in localized failure of mineralization of the growth plate. At necropsy, a cone of cartilage ("cartilage plug") projecting into the long bone (diaphysis) is found. It usually occurs in the drumstick-bone (proximal tibiatarsii). It is more prevalent in males than females, around 12-20 weeks of age. There are several factors that influence the incidence of TD in a flock. Some of the factors involved are: genetics, growth rate, electrolytes, calcium, phosphorus, and mycotoxins. It has been associated with genetics and/or diets for rapid growth. Improper calcium/phosphorus ratios in the diet have been implicated. The exact cause is unknown. In broilers, mycotoxins (such as *Fusarium roseum*) have been implicated as a cause. a 1994 field study (Po Sci 73:1254-9) found a TD incidence of about 70% in 14-week-old turkeys and about 80% in 15-week-old turkeys, with a tibial growth plate OM incidence of about 1%.

Toe Conditioning: New technology for toe conditioning utilizes the Nova-Tech Poultry Services Processor Microwave Treatment (Willmar, MN, USA). This process is conducted at day-of-age in the hatchery to the tip of the toenails of the 3 large digits (toe). It destroys germinal tissue at the tip of the toe. Toenails that are conditioned with the fully automated microwave procedure subsequently drops-off after 7 to 10 days of age. Toe conditioning is performed, upon customer's request, using specialized equipment, most commonly the Nova-Tech Services Processor, by trained personnel and closely monitored by a quality assurance inspector. The Nova-Tech Engineering, Inc and its patented Claw Treatment Process equipment was initially installed in a select few hatcheries in the States in 1994. Currently the vast majority (>90%) of all turkey hatcheries in the States and Canada use the Nova-Tech Claw Treatment Equipment.

Turkey Coronavirus (TCV), also known as Bluecomb disease or mud fever, is a highly infectious and acute enteric (intestinal) viral disease of turkeys. Serologic diagnostic tests for TCV are available from several of the State poultry diagnostic laboratories. TCV is a significant economic problem, mainly due to poor flock performance, causing financial losses for both growers and processors. The incidence of TCV was less than the previous year in the Southeast despite a few cases localized to one small area. A couple localized areas in the lower Midwest and Southeast were recently diagnosed after being negative for nearly 2 and 1 year(s), respectively. Coronavirus was recently reported to be associated with enteritis in Midwest.

Special acknowledgments to Mr. Greg Hansen, Drs. Tasheen Aziz, Yan Ghazikhanian, Wayne Williams, Keith Turner, Andy Yersin, Andrea Miles, Cheryl Hall and industry veterinarians and technical specialists who contributed to this presentation.

Notes:

The Very Virulent Infectious Bursal Disease Virus

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The appearance of the very virulent infectious bursal disease virus (vvIBDV) in Europe in 1987 was a classic story of the evolution of a pathogen leading to the emergence of a devastating disease. After its appearance in Europe the disease spread to the Middle East, Asia and Africa. The disease was confirmed in South America and the Caribbean in 1999. Currently, North America, Australia and New Zealand are the only areas where the disease has not been diagnosed.

The Disease

The disease caused by the vvIBDV has an incubation period of 3 to 4 days. It is acute and the birds are usually depressed and reluctant to move with ruffled feathers and diarrhea. The lesions are very prominent and include muscle hemorrhage and swollen hemorrhagic bursas and spleens. The onset of the disease is rapid and the morbidity is high. Mortality is high but it varies dependent as the antibody level. With moderate levels of antibodies, mortality of 30% and 70% in broilers and layers respectively is common. In birds with no antibodies, mortality could reach 90 to 100%.

The Virus

Antigenically, the vvIBDV is similar to the classic strains of serotype 1. Markers for virulence of IBDV have not been described, hence, *in vivo* testing is needed for defining pathogenicity of the virus. Sequencing studies on the vv viruses indicated the presence on the VP2 of four specific amino acids (aa) in all vvIBDV. In is thought that these aa represent evolutionary markers rather than virulence markers.

Diagnosis

The vvIBDV replicates in embryonating eggs but is very difficult to propagate in tissue culture. Extensive manipulations have been used to induce adaptation to cell culture. Classical diagnostic tests are useful for detecting the virus and lately the use of the RT-PCR has become widespread. That test proved to be more sensitive than all other routinely used tests. To differentiate strains of the virus, restriction enzyme analysis is used. Specific enzymes have been used to differentiate the vvIBDV.

Disease Prevention

Vaccination is the main tool used to prevent the disease. Vaccination programs for the vvIBDV are usually very aggressive with a main goal of inducing a high level of antibodies. Since the vvIBDVs are antigenically similar to the classic strains of the virus, currently available commercial vaccines made of the classic or variant strains are effective against the vvIBDV.

In summary, the vvIBDVs cause a devastating disease in chickens. The disease has not been detected in the USA but it was diagnosed recently in the Caribbean. The poultry industry should be educated on the dangers of this disease. Diagnostic tools to differentiate the vvIBDVs from other viruses are available. Currently available commercial vaccines are effective in inducing immunity against the vvIBDV, but aggressive vaccination strategies should be followed to induce high levels of antibodies.

Notes:

Retroviruses and Marek's Disease Virus

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Avian retroviruses, namely avian leukosis and reticuloendotheliosis viruses and Marek's disease virus (MDV), an alphaherpesvirus, are the most common naturally occurring viruses associated with economically important virus-induced neoplastic diseases of poultry (5,18,19,20). This review is primarily aimed at addressing current status and future challenges regarding control of these three important viral infections of poultry.

Avian leukosis virus

Avian leukosis virus (ALV) is a member of leukosis/sarcoma (L/S) group of avian retroviruses viruses that can cause tumors and other production problems in chickens (5, 18). Although ALV is capable of inducing a variety of neoplastic conditions in chickens, lymphoid leukosis, a B-cell lymphoma affecting primarily the bursa of Fabricius and visceral organs is the most common form of leukosis that arise from infection with ALV. However, with the recognition of subgroup J ALV infection in the early 1990's, myelocytomatosis, has emerged as a neoplastic condition that is frequently detected in ALV-J- infected meat-type chickens (5,18). Like other retroviruses, ALV mutates at a high rate and can recombine with endogenous (subgroup E ALV) elements resulting in new recombinant ALVs (5,18). These endogenous subgroup E ALV elements not only contribute to recombination, but also can interfere with diagnosis and control of ALV infection. Recombination can also occur between members of two different subgroups of exogenous ALV. Recent laboratory observations provided evidence for recombination between subgroup A and J ALV (ALV-A/J), a recombinant ALV with the envelope of subgroup A and LTR of subgroup J; this recombinant ALV resulted from passing ALV-J in cells expressing subgroup A envelope (16). Recombination between members of two subgroups of ALV can also occur under field conditions, resulting in the emergence of a natural recombinant virus. Recently, an ALV-B/J, a recombinant ALV with envelope of subgroup B and LTR of subgroup J was isolated from commercial layers affected with myelocytomatosis (9).

Natural infection with ALV has been known to cause significant economic losses in commercial layers and breeder flocks due to mortality and lower productivity. As a potential contaminant of live-virus vaccines of poultry, ALV can also cause significant losses if contaminated vaccines were used in susceptible flocks. Most recently, a subgroup A ALV was isolated from commercial Marek's disease vaccines; however source of such contamination has not been determined yet (6,7). To date, no vaccines are available for control of ALV infection. Thus, eradication of virus infection at the primary breeder level is and has been the principal method for controlling ALV infection in chickens. The new advancements in knowledge regarding molecular characteristics of

ALV genome, development of highly specific reagents such as monoclonal antibodies and other technologies such as cloning of viral genes have contributed significantly to improved diagnosis and control of ALV. Clearly, diagnosis and control of re-emerging recombinant ALV that cause disease in chickens represent new challenges that must be addressed in order to reduce losses from future outbreaks with previously unrecognized subgroups of ALV.

Reticuloendotheliosis virus

Reticuloendotheliosis virus (REV) is an avian retrovirus unrelated to the L/S group of viruses. REV infects chickens, turkeys, ducks, geese, pheasants, quail, and probably many other avian species (18,19). The most common clinical diseases induced by REV are chronic lymphomas and an immunosuppressive runting disease. Although REV is widespread, REV-induced clinical disease is infrequently diagnosed in commercial flocks (18,19). All isolates of REV are remarkably uniform in antigenicity and have similar structural and chemical characteristics (19). Although REVs are known to belong to a single serotype, three subtypes were identified on the basis of neutralization tests and differential reactivity with monoclonal antibodies (19). Also, unlike the case with ALV, no endogenous REV elements have been identified.

Although losses in REV affected flocks can be significant due to tumor mortality and or immunosuppression (1,18,19), the principal economic concerns of REV infection are: a) as contaminants of live-virus vaccines of poultry, and b) as a barrier to export of breeding stock to certain countries. To date, no vaccines are available for control of REV infection and unlike the case with ALV, no method has been routinely used by industry to control REV infection in commercial turkey and chicken flocks. Current information on partial or complete REV genome insertion in large DNA avian viruses such as Marek's and fowlpox viruses (4,8,12,13,14,15,17) have indicated the need for further studies to determine the role of such insertion in the epidemiology of REV as well as the pathogenicity of these large DNA viruses. Contamination of vaccines, partial or complete insertion of REV genome in other viruses and developing new control methods represent important challenges that must be addressed in order to develop effective strategies for control of REV infection in poultry.

Marek's disease virus

Marek's disease (MD), a T-cell lymphoma of primarily chickens is caused by a highly cell-associated alphaherpesvirus (20). The disease is and has been controlled since early 1970s by use of conventional vaccines. During the last three decades, research on MD has resulted not only in improved conventional vaccines, but also in improved methods of vaccination (embryo vaccination). Good biosecurity practices and host genetic resistance are also recognized as important factors in implementing any strategy for control of MD. However, despite widespread use of vaccines and development of new methods of vaccination, economic losses from mortality of layers and breeders and condemnation of broilers continue to occur (18,20). In recent years, MD has been diagnosed in commercial turkey flocks in Germany, France and Israel (2,3,10,11,18,20), suggesting that the host range of MDV has apparently expanded to include turkeys. More studies are needed to understand factors that lead to MD outbreaks in commercial turkeys.

The fact that MDV continues to mutate to greater virulence, reducing the effectiveness of many existing vaccines (20) is a major concern to the poultry industry. Obviously, an important challenge regarding control of MD in the future is developing new strategies to control losses caused by new emerging MDV pathotypes. Development of vaccines that can interfere with replication and shedding of MDV, and understanding role of host genes involved in resistant to MD will undoubtedly improve our ability to implement a better strategy for control of MD in the future.

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Notes:

Emerging Bacterial Diseases

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I am unaware of new emerging bacterial diseases of poultry arising from some exotic or native avian species although prevalence of certain bacterial species has been altered due to changes in management practices or to regulatory restrictions on use of antimicrobial drugs for growth promotion. This last change will undoubtedly be compensated for in the short term by increased therapeutic use of antimicrobial agents with the consequence of a continued increase in frequency of drug-resistant enteric bacteria capable of being transmitted through the food chain to human consumers of poultry products. Since there is an increasing societal concern for food safety that is magnified by our concern for bioterrorism threats, there is likely to be increased surveillance of poultry products for the presence of microorganisms harmful to humans and likely future regulations to diminish their presence. I will thus discuss this afternoon some of these issues and suggest means by which these and other infectious disease problems in poultry might be prevented to enhance food safety by reducing populations of human enteropathogens and commensal bacteria including those harboring drug-resistance genetic information. A long-term objective will therefore be to create a mostly drug-free environment within the poultry industry.

Numerous bacterial species that contain representative strains capable of causing human disease are also prevalent in poultry. Similarly, some bacterial species that are capable of nosocomial infection as opportunistic pathogens in humans are also commensals, mostly without ill effect, in poultry. The realization of and understanding of these problems were based on an initial awareness of colonization of poultry by *Salmonella enterica* serotypes that were early recognized as potential human pathogens to cause gastroenteritis and diarrhea (3,12). With passing time, and an awareness of the existence of numerous *Escherichia coli* pathovars causing diarrheal diseases and extra-intestinal infections, especially of the urinary tract, in humans (10,15,23), it was observed that unique strains of *E. coli* existed that infected poultry, mostly by the respiratory tract to cause airsacculitis, pneumonia, and septicemia (5). Since APEC infections caused mortality and carcass condemnation and presence of *Salmonella* had a negative impact on consumer acceptance and popularity of poultry products, many changes in management procedures during production and processing have been made with a very significant impact on reducing the prevalence and consequence of *Salmonella enterica* serotypes and *E. coli* pathovars in poultry. In spite of these population density decreases, it would appear that the frequency of these enteric pathogens and commensal non-pathogens possessing drug resistance and especially multiple antibiotic resistance genetic elements has continued to increase. This has intensified concerns for the impact of antibiotic usage in agriculture on human health (28) because of the demonstrated ability of these bacteria to be transmitted from poultry production facilities through the processing plant to infect humans, who unfortunately have to bear some responsibility for their poor food handling procedures that often are responsible for their infections. More recently, it became evident that

Campylobacter jejuni (1,25) and *Listeria monocytogenes* (4,8,25) were essentially everywhere and thus present on most poultry carcasses leaving processing facilities to be marketed in grocery stores. Fortunately, *Listeria* does not seem to be transmitted significantly to humans from poultry but this is not the case for *C. jejuni*, which now accounts for the majority of enteric infections in the U.S. and a number of deaths close to the number caused by *Salmonella enterica* infections (19). Even more recently, it has become apparent that *Enterococcus* species present in poultry (13,14,17) can also be transmitted through the food chain to humans. This has raised concern because of multiple drug resistance and the presence of *Staphylococcus aureus* strains in poultry (16,27) that might also be transmitted through the food chain. This concern is magnified by the fact that the most severe hospital-acquired infections are often due to infections with multiple drug-resistant *Enterococcus* species and *S. aureus* (6,9).

Scandinavian countries have worked diligently to reduce antibiotic usage in rearing of poultry and other farm animals so as to lessen selection pressure for proliferation of drug-resistant bacterial strains that could potentially be transmitted through the food chain to humans (29). Although success has been achieved to some extent in reducing frequencies of drug-resistant microorganisms, other consequences in this change in management have arisen that potentially diminish somewhat the productivity of the poultry- and swine-producing industries. Thus, there has been an increased prevalence of *Clostridium perfringens*, mostly Type A, and an increased incidence of necrotic enteritis (11). It is apparent, that this increase prevalence of *C. perfringens* is a consequence of diet with cereal grains such as wheat barley and rye enhancing *C. perfringens* colonization (21) in contrast to diets with corn and soybeans that does not provide such a benefit to this bacterial species (2). Nevertheless, the antibiotics used for growth promotion seem to have a most pronounced effect on reducing *C. perfringens* population densities in poultry (24) such that elimination of growth-promoting antibiotic usage is likely to increase adverse consequences due to the presence of *C. perfringens*. The α-toxin produced by essentially all strains of *C. perfringens* can cause a decrease in microvillus length and a thickening of the intestinal mucosa, especially in the ileum, to result in a decreased efficiency at nutrient uptake (26). Thus the effect of *C. perfringens* may be subclinical but significantly reduce feed conversion efficiency and growth rate with an increase in time to achieve market weight. It is also apparent that many of the coccidiostats used to control *Eimeria* infections are also effective in reducing *C. perfringens* population densities in the intestinal tract of chickens (7). Thus development of effective vaccines against *Eimeria* species with a reduced use of coccidiostats coupled with an imposed reduced use of subtherapeutic antibiotics will have the collective impact of further enhancing *C. perfringens* population densities with a more significant reduction in bird performance.

Although I believe that most of the blame for nosocomial infections of admitted patients with antibiotic-resistant opportunistic pathogens in hospital settings is due to poor practices in the medical profession and poor hospital infection control management practices rather than due to antibiotic usage on the farm, I have to acknowledge as a geneticist that reducing selection pressure for the multiplication of drug-resistant bacteria is in the long-term, in the best interests of both agriculture and medicine. I therefore accept attempts to reduce the

selection pressure on the farm, which is far removed from the hospital settings where the problems are occurring, but endeavor to develop strategies to improve animal performance without reliance on drugs and with a concomitant enhancement of food safety by diminishing the probability of transmitting human commensals and enteric pathogens through the food chain to the consumers who we hope will reward the poultry industry by further increasing consumption of poultry products as a major source of protein. It would also help, if we could be more educational in encouraging safer food handling practices.

Fifteen years ago, we constructed an attenuated strain of *S. typhimurium* that was finally APHIS licensed in 1998 to control *Salmonella* infection in broilers and more recently to control *Salmonella* infection in layers. Although this vaccine, and others like it, have the potential to very much diminish if not eliminate *Salmonella*, when flocks are not initially heavily contaminated, it is seldom used and there have been no enhancements in food safety as a consequence of its development. While this has been frustrating to me, I am aware that poultry is a commodity and a penny a bird for a vaccine translates to $80 million a year and that is real money. Thus, a vaccine that does not result in performance enhancement is of no benefit to the producer, especially when the FDA permits up to 20% of carcasses to be contaminated upon completion of processing and only imposes penalties when this limit is exceeded three times in a row. As stated above, much improved management practices have kept the prevalence of *Salmonella* well below this 20% limit in the vast majority of production facilities, which are very well managed with regard to *Salmonella* control. It therefore becomes evident, that use of any food safety vaccine is dependent upon the ability of that vaccine to provide economic benefit to the industry enhancing productivity. Before addressing a potential means to accomplish this objective, let me describe some new technologies to further enhance the safety and efficacy of live bacterial vaccine vectors to control bacterial, viral and parasitic infections in poultry.

Many of the traditional means to attenuate *S. typhimurium* cause strains to be intolerant of stresses such as exposure to acid, bile, increased osmolarity, iron and other ions and/or diminish the ability of strains to attach to and invade the gut associated lymphoid tissue (GALT). Thus, while conferring attenuation, immunogenicity is significantly decreased. It would be far superior if vaccine strains would exhibit wild-type abilities to withstand all stresses and host defenses encountered following oral or intranasal immunization and would exhibit wild-type abilities to colonize and invade host lymphoid tissues before displaying an attenuation phenotype resulting in inability to cause disease symptoms. In addition, it would be desirable if the *S. typhimurium* vaccine strain maximized immune responses to immunologically related/cross-reactive antigens such as the LPS core polysaccharide, many outer membrane proteins (OMPs) and iron-regulated outer membrane proteins (IROMPs) and diminished immune responses to serotype-specific antigens such as LPS O-antigen and the phase 1 and phase 2 flagellar antigens. This would result in a vaccine that would induce maximal immune responses to antigens that would confer cross-protective immunity to *S. enterica* strains of diverse serotypes and also to a substantial number of *E. coli* serotypes. We have accomplished these objectives using a number of different strategies. We constructed five different types of mutational alterations that impose regulated delayed attenuation phenotypes such that bacteria at the

time of vaccination exhibit wild-type attributes with regard to responses to all stresses and abilities to attach to and invade the GALT and then after efficient colonization of internal effector lymphoid tissues undergo a transformation to display an attenuation phenotype and become unable to induce disease symptoms. In some cases, we accomplish this by using mutations to enable synthesis of LPS O-antigen when the vaccine strain is grown in the presence of mannose whereas the synthesis of O-antigen ceases in vivo because of unavailability of free mannose (18,22). In other instances, expression of virulence attributes is dependent upon the presence of arabinose and after immunization and colonization of lymphoid tissues these genes are no longer expressed due to the total absence of free arabinose in vivo. Further modifications to diminish immune responses to serotype-specific antigens have been achieved by deleting the sequences encoding the antigenic variable portions from the two genes encoding flagellin. In this case, the N- and C-terminal domains of flagella are still present to interact with TLR5 to recruit/stimulate innate immunity. This *S. typhimurium* strain induces antibodies that react with surface antigens on a great diversity of *S. enterica* and *E. coli* serotypes. This vaccine is being further modified to express recombinant protective antigens from other bacterial species such as *C. jejuni*, *L. monocytogenes* and *C. perfringens*. We make use of balanced-lethal host-vector systems (20) in which the plasmid vector encodes an enzyme involved in the synthesis of an essential cell wall constituent such as diaminopimelic acid (DAP), D-alanine or muramic acid and introduce into the chromosome of the *S. typhimurium* a deletion mutation for the gene encoding this enzyme involved in DAP, D-alanine or muramic acid synthesis. The use of these host-vector systems ensures that the vaccine strain is fully antibiotic sensitive and expresses no antibiotic resistance traits. These plasmid vectors have been further modified to deliver protective antigens by two different secretion routes and by a regulated delayed lysis in vivo phenotype to release a bolus of protective antigen. This regulated delayed lysis leads to the total demise of the vaccine population and thus confers total biological containment. We are in the process of making a recombinant vaccine against the *C. perfringens* α-toxin and have made progress in constructing various recombinant vaccines against *Eimeria* sporozoite and merozoite antigens. We are a long ways from success but have considerable confidence in the host-vector systems that we have so far developed and are optimistic that we can control both *C. perfringens* and *Eimeria* to reduce the need for antimicrobial drugs in poultry rearing and enable use of a vaccine to benefit the industry and at the same time enhance food safety.

The research that will be described has been generously supported by the USDA, NIH and the Ellison Medical Foundation.

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Notes:

Blackhead Disease in Turkeys and Chickens as a Re-emerging Disease

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Prior to the availability of effective treatment, blackhead (*Histomonas melelagridis)*, was responsible for more losses in turkeys than any other disease, and led to abandonment of turkey rearing in parts of the USA (2). With effective control measures in the 1960s, reported cases of blackhead dropped precipitously and remained low until the 1990s. Widespread clinical blackhead cases in broiler breeder pullets in the 1990s, and the sudden spate of cases of blackhead in turkeys in California, Arkansas and North Carolina in recent years, has brought new research attention to a long-dormant field.

Interaction of blackhead with Coccidia: Concurrent inoculation with cultured *Histomonas* and a mild dose of *E. tenella* causes an increase in the number and severity of liver lesions in chickens (6). This effect was not ameliorated by anticoccidials or antibiotics (3). In turkeys, the cecal coccidia (*E. adenoeides*) had no effect on infection or development of lesions. Studies with *E. adenoeides* in chickens were also negative.

Epidemiology of blackhead: Given the explosive spread through flocks with mortality of 80-100% in 2 weeks, it seemed apparent that some mechanism of exposure other than cecal worm eggs was responsible for bird-to-bird infection. To investigate this, we placed turkey poults on fresh litter in pens (no vectors present) and infected 10% or 25% with *Histomonas* from cultures. Within 30 days, all of the birds had died or had severe lesions of blackhead (4). To investigate this further, we attempted to infect turkey poults by oral inoculation of cultured *Histomonas*, or by feeding of droppings from infected birds. These attempts were unsuccessful. However, when we applied drops of cultures to the vent and stimulated "cloacal drinking," the poults became infected. We now believe that some form of this process is responsible for natural exposure and rapid spread of blackhead within turkey flocks (5).

Laboratory models for blackhead research: After the observation of lateral dissemination of infection in floor pens, we attempted similar studies with 2-wk-old turkeys in battery cages. The floor screens were covered with heavy paper for several days to allow some normal build-up of droppings, but it is not know whether this was important. When 25%, 37.5% or 50% of the poults were inoculated with histomonads from the cecas of sick turkeys, they became infected and suffered high mortality. The uninoculated birds in the same cages also became infected and suffered high mortality or severe lesions. There was no difference in the number of uninoculated birds becoming infected in the 3 exposure levels. If the inoculated birds were removed after only one day of exposure, 20% of the uninoculated birds became infected. If the inoculated birds were left in the cage for 2, 3, or 4 days, more than 90% of the uninoculated birds became

infected. This demonstrates that transfer from infected bird to uninfected bird is rapid, and that birds are highly contagious to other birds only 2 days postexposure.

Unusual findings in field outbreaks: The unexpected virulence of *Histomonas* in chickens, and reports of atypical lesions suggest the emergence of new strains. Older literature suggests lesions only in the liver and ceca. However, a recent report from California (Prasad, Pers. Com.) noted prominent lesions in the bursa (26%) and kidney (16%), and occasional lesions in the lung, proventriculus and pancreas.

Future control of blackhead: Presently no products are approved for treatment of blackhead. Histostat is available for prevention. After outbreaks, questions focus on disinfection of farms and litter treatment. The results of recent studies suggest that outbreaks on turkey farms come from chance introduction of infection from outside, and are able to spread rapidly through the flock without the use of vectors. Thus, it would not seem important to use extreme measures to disinfect the premises. In chickens, which are better hosts for cecal worms, the floors are universally contaminated (1), and efforts can be aimed at litter management and worm control for reduction of blackhead exposure.

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Notes:

BIOTERRORISM

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As we all know, terrorism can take many forms to inflict the desired damage. It can be directed against any part of a society: its people, its property, its treasured landmarks, its transportation, or its food supply, to name a few.

I will confine my comments today to a relatively narrow, focused part of the terrorism spectrum called bioterrorism and only to a portion of that. I will not speak about the contamination of human food or water supplies with infectious disease agents or toxins. Neither will I address the covert use of highly contagious infectious agents of human diseases to cause illness and death among the population. I will confine my comments to poultry bioterrorism which I would like to define for the purpose of this discussion as "the intentional introduction of causative agents of diseases of poultry with the goal of causing economic losses, negatively impacting food supplies and ultimately creating fear and hardship among those who rely on poultry as a source of food or as a livelihood".

It is impossible for any of us to say with much confidence, what disease agents would be used should a bioterrorist attack occur or how those agents would be introduced. It borders on being reckless to speculate about possible diseases that would likely be used and against what segment of the industry. That said, however, it may be beneficial to select a candidate disease virus such as highly pathogenic avian influenza (HPAI) to facilitate this discussion. Some HPAI isolates can, without manipulation, inflict serious losses in poultry with the mortality in some naturally infected flocks approaching 100%. The virus replicates to very high levels when injected into the allantoic cavity of 10 day-old embryonating chicken eggs, maintaining infectivity in those fluids without refrigeration for extended periods. The serious damage that HPAI can inflict on a poultry industry is well known in many parts of the world, making it an ideal and perhaps even likely disease agent for poultry bioterrorism.

What segment of the poultry industry would be the most likely to be attacked? By virtue of their numbers, the turkey or broiler grow-out flocks would be the easiest targets and perhaps the most likely. Because of the generally lax attitude in the industry about many biosecurity practices during the lulls between serious disease scares, a few well-placed disease introductions would quickly spread within and maybe even between the different poultry companies. This would likely occur before the industry knew that disease introductions had been made. Concurrent introductions of other diseases such as viscerotropic velogenic Newcastle disease (VVND) would create confusion and uncertainty in the diagnosis. Because the introductions would be at the grow-out level, there could soon be a negative impact on poultry meat supplies, depending upon how

extensive the introductions and how rapidly the disease spread. In addition to this negative impact on poultry meat supplies, because of disease prevention and control restrictions, both domestic and foreign markets would likely be lost.

The very large multi-million bird table egg operations would be ideal targets for someone wishing to have a rapid negative impact on the food supply. Even though hens are confined in small groups in cages, HPAI introduced into a cage near the air intake of each in-line house would soon make its way through the complex, assisted by personnel, flies, airflow and feed chains. Layers that did not die would likely cease to produce eggs for several weeks and probably would never return to normal production levels.

A bioterrorist attack on a primary breeder flock would not exert its negative effects on the consuming public as quickly as would attacks on production flocks. The long term effects could, however, be very significant and costly. On the positive side, the breeder companies are internationally based and could probably relocate breeding stock to expedite a more rapid recovery of that very valuable resource. Attacks on multiplier breeder flocks of any of the three poultry categories would have serious consequences in that there is generally very little excess hatching egg production. If grow-out flocks are lost, they can be soon replaced after the cleanup as long as replacement chicks and poults are available. If multiplier flocks are lost, it will be many months before they can be replaced to produce fertile eggs, even if the genetic stock is available.

I don't believe that I need to insert additional speculation into this discussion about who would want to attack us and why. The events of the last few years have likely created enough paranoia in each of us to last a lifetime. Unfortunately, the concern may be very real and not paranoia after all. Only time will reveal the truth to us. The terrorists that would want to carry out such attacks can come from other countries or from within these borders. They can be motivated to inflict such harm by political, social, environmental, religious or economic concerns or by combinations of factors. Attacks could simply be an extension of the current terrorist conflict. There are fanatical animal rights advocates that have already committed terrorist acts against research laboratories, environmental activists that drive metal spikes into trees to keep them from being harvested and those than riot to disrupt economic summits. There is no shortage of individuals who may wish to use bioterrorism to bring down the poultry industry.

I am of the opinion that we will not likely be able to prevent all such attacks by any individual or groups of individuals who are truly dedicated to the mission of inflicting damage to the poultry industry using bioterrorism. The opportunities for such attacks are too many and they are too easy to accomplish without personal risk to the terrorist. Because we are so vulnerable to such attacks, It is my sincere belief that we must establish a biosecurity program in the poultry industry that will have a good chance of confining any such covert disease introductions to the initial flocks where they are introduced. Although not totally preventing all losses, such measures will likely prevent what could evolve into an absolute disease disaster.

Impossible to accomplish that level of biosecurity? No. Difficult to do? Yes. Considering the possible, if not probable, scope and severity of the consequences of any such attacks without biosecurity barriers in place, we have no choice but to get on with it. A Biosecurity educational CD prepared by Dr. J.P. Vaillancourt and Mr. Gene Lambert with help from numerous experienced individuals was funded by the US Poultry & Egg Association. A free copy can be requested at www.poultryegg.org . The CD is a good place to begin. It is user-friendly and contains a wealth of readily available information that is important in setting up a biosecurity program.

Notes:

Preparedness and Response to Emerging Diseases – National Perspective

Dr. Jack Shere
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Notes:

Preparedness and Response to Emerging Diseases – State Perspective

Dr. John Enck Jr.
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Avian Influenza in PA

- 1983-84 - H5 N2 - 17 million birds, 452 flocks
- 1996 – 1998 - H7 N2 - 2,623,116 birds, 24 premises comprising 47 flocks
- Nov. 2001 – Jan. 2002 – H7 N2 - 170,500 birds on 9 premises
- Average 13 investigations a year for non H5, H7 AI strains
- February 2004 – H2 N2 – 462,000 birds, one complex
- May 2004 – H2 N2 – 879,400 birds, one complex

Surveillance in PA Industry

- AI Monitored Flock Program
  - 331 flocks –tested monthly
- Regular Testing
  - 21 Poultry Auctions, 2 Live Bird Markets
- Ongoing Laboratory Diagnostics
  - 211,000 tests last year
- Meat Birds and Commercial Egg Layer Surveillance
  - 700 flocks at slaughter
- NPIP

MANAGEMENT OF AI QUARANTINED FLOCKS

Indications for Depopulation of Poultry Following Confirmation of Index Case

- Suspect or positive test
- Clinical signs or pathology compatible with AI in birds
- Epidemiologic evidence that the flock was exposed
- Compromised or inadequate biosecurity practices on site

Actions Taken After Detection of AI

- Quarantine infected or exposed flocks
- Depopulate infected birds
- Depopulate potentially exposed and at-risk birds to decrease the number of susceptible birds that could continue spreading the virus
- Delay repopulation until C&D is complete
- Control movement of birds
- AI Area of Operations

H2 N2 - Chicken Disease? OR Political/Economic Nightmare

- H2 N2 diagnosis in chickens – no problem
  - Not recognized by management
  - Low mortality
  - Little egg production decrease
  - Not all houses in the complex sero-converted

H2 N2 Big Problem

- Twenty some people had died recently on the pacific rim from "avian flu."
- CDC website – 1957-58, "Asian flu," [A (H2N2)] causes about 70,000 deaths in the United States.
- PA Department of Health wants eggs held on 462,000 birds until FDA issues an "OK."
- USDA will announce that we have H2N2 in 15 minutes so Russians know we are not part of Delaware case.

Enhanced Biosecurity for PA Poultry Producers

- Prevent wild bird poultry interaction- keep waterfowl from ponds
- Provide clean clothing for essential workers and allow only disinfected vehicles on farms
- Do not loan or borrow equipment
- Do not bring dirty coops from LBM's on farm

Infectious diseases can be spread from farm to farm by:

- Introduction of diseased birds
- Shoes and clothing of visitors or caretakers who move from flock to flock
- Contact with other inanimate objects (fomites) that are contaminated with disease organisms
- Carcasses of dead birds that have not been disposed of properly
- Impure water, such as surface drainage water
- Rodents, wild animals and free-flying birds
- Insects
- Contaminated feed and feed bags
- Contaminated delivery trucks, rendering trucks, live hauling trucks
- Contaminated premises through soil or old litter/manure

Lessons Learned…

- Ongoing Surveillance: Established AI Surveillance Program
- Biosecurity: Through education and commitment
- Disease Signs (Symptoms): Highly pathogenic AI viruses have more consistent presentation similar to Exotic Newcastle Disease (VVND); mildly pathogenic AI viruses appear like any poultry respiratory disease or disease decreasing egg production
- Early Detection: Have diagnostic capabilities, producer education and cooperation (identify first case)
- Quarantine Immediately: Strengthen biosecurity with personal epidemiological evaluation with grower and family very soon after each case diagnosis
- Aggressive Action: Have management plans prepared in cooperation with Prefecture Governments, universities, environmental regulators and the poultry industry
- Indemnity fund: Having immediate funds available to support depopulation and help with grower incomes
- Depopulation Methods: Employ methods of depopulation which prevent further spread of the virus; consider composting in the house with floor birds
- Avoid Repopulation: Do not restock naïve poultry in outbreak area until likelihood of re-infection is strictly minimized
- Sentinel Surveillance: Place sentinel birds in houses where birds have been exposed if the decision is made to maintain seropositive birds; sampling of mortality for a period is advised
- Be Prepared: The specific nature of the outbreak virus and the type of birds infected or lesions noted will dictate the particular applications of control measures and subsequent surveillance details (types of tests, sample size, frequency of application)

Changes Needed to LBM System

- Wholesale buyers and growers agree to grow flocks to a particular age and weight with many fewer individual load-outs
- Efforts to improve and maintain biosecurity among load-out crews (cloths/footwear/vehicles)
- At Risk: Introduction and spread of infection to commercial poultry, economic losses as a result of quarantines, loss of international poultry trade

Questions?

- Are non H5 and non H7 strains in chickens reportable? Any species?
- What strains need reported to the department of health?
- When do you notify the department of health?

Notes: