Managing Respiratory Diseases from the Field, Diagnostic Lab and through Research: Emerging and Re-emerging Challenges

AAAP Symposium Program

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Table of Contents

American Association of Avian Pathologists
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2008 AAAP/ ACPV Symposium
Managing Respiratory Diseases from the Field, Diagnostic Lab and through Research; Emerging and Re-emerging Challenges
July 20, 2008

Time	Topic	Speaker
8:00-8:05	**Welcome/Housekeeping**	Mediators: Dr. John El-Attrache/ Dr. Babak Sanei
	Diagnostic Lab and Field	
8:05-8:25	Overview – Perspective from the Field "What does the field expect from the Diagnostic Lab"	Dr. John Smith
8:25-8:45	Overview – Perspective from the Diagnostic Lab "What does the lab expect from the Field Clinicians"	Dr. Jose Linares
	Infectious Bronchitis	
8:45-9:10	Field Perspective – Vaccination Programs and Adjustments	Dr. Phil Stayer
9:10-9:35	Changes in the future – "Challenges from variants viruses – "Can we learn from the past?"	Dr. Jack Gelb
9:35-10:00	**Break**	
	Infectious Laryngotracheitis	
10:00-10:25	Use of vaccines in the control of ILT outbreaks in broilers – vaccination in the face of ILT infection	Dr. Guillermo Zavala
10:25-10:50	ILT Diagnostics – Interpreting PCR, Histopathology and Virus Isolation results	Dr. Maricarmen Garcia
10:50-11:15	Field perspective – Regional Report Southeast "Epidemiology of ILT outbreaks and application of GIS"	Dr. Louise Dufour-Zavala
11:15-11:40	Field perspective – Regional Report West "How are ILT breaks handled?"	Dr. Stewart Ritchie
11:40-12:05	Field perspective – Regional Report International "How are ILT breaks handled?"	Dr. Mariano Salem
12:05-1:05	**Lunch**	
	Mycoplasma gallisepticum	
1:05-1:45	Field and Lab Perspectives- How are MG breaks handled and diagnostic tools available for diagnosis and characterization?	Dr. Stanley Kleven
	Avian Influenza	
1:45-2:10	Experimental Vaccination Programs -DIVA	Dr. David Suarez
2:10-2:40	The Role of Vaccines and Biosecurity in Control of H3N2 Swine Influenza Infection in Turkey Breeder Flocks	Dr. Becky Tilley

Speakers Information

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We want to thank all of the speakers for their cooperation and contributions.

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DIAGNOSTIC LAB AND FIELD: OVERVIEW PERSPECTIVE FROM THE FIELD

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When a field clinician submits a specific sample to a laboratory for a specific set of requested tests, the expectations are clear. He or she expects complete, accurate, reproducible results reported in a clear and timely manner; confidentiality; and clear, accurate, and timely billing and accounting. The resources needed to provide the level of service expected by today's practitioner are set forth by accrediting agencies such as the World Organization for Animal Health (OIE) in the *OIE Quality Standard and Guidelines for Veterinary Laboratories: Infectious Diseases, 2008* and the American Association of Veterinary Laboratory Diagnosticians' *Essential Requirements for an Accredited Veterinary Medical Diagnostic Laboratory*. The resources needed to meet our expectations include competent, trained, and proficient technicians; a rigorous quality control system; adequate physical resources such as buildings, equipment, and reagents; excellent document control and records systems; and the use of the most up-to-date, validated, and accepted test methods appropriate to the assays requested. If a test cannot be performed adequately, it should not be offered. If a test is not available but clearly indicated, the laboratory, in consultation with the client, should forward the sample to a laboratory that can conduct the test and report the results to the client with full disclosure of the source of the results. Laboratory diagnosticians should be willing to seek a second opinion when needed.

The relations between field clinicians and diagnostic laboratories in the commercial poultry industry are probably a bit more collegial than in many other areas of veterinary medicine. We are a small group, we frequently know each other personally, and the working relationship is closer. There are essentially no poultry referral practices analogous to the referral practices available to companion and even some other food animal practitioners. The poultry field clinician is the expert, and higher levels of expertise are found only in the academic departments and laboratories which are not practices per se. The laboratory therefore becomes a referral center of sorts for the poultry clinician. The relationship goes far beyond submitting blood or serum for a specific list of serological assays, fixed tissue for histopathology with a desire to rule in or out a specific differential, or fresh tissues or swabs for isolations and identifications. Rather than a single diagnosis on a single case, we are frequently looking for help in the diagnosis and management of a recurring problem or an outbreak. The expectations of the laboratory go beyond the timely reporting of accurate results, and we are looking for a two-way partnership in solving a problem.

In the context of this relationship, the field clinician may submit specific tissues with specific tests requested, but may also desire the laboratory clinician to consider the history, signs, and lesions reported and select additional tests for those specimens. The field clinician will often submit or direct the submission of live, moribund, or (hopefully) fresh dead birds. In such cases he/she expects a thorough necropsy examination with attention to detail, the selection and proper

collection and processing of appropriate specimens for further testing, and the ordering of appropriate tests on those specimens. Often, the field clinician desires a second set of eyes to examine the cases he/she is encountering: are we missing significant lesions, have we overlooked an important differential diagnosis, are there other tests that are indicated? Depending on the specific relationship, the field clinician may expect the laboratory diagnostician to preemptively perform the indicated tests, or discretely communicate those suggestions. Most field clinicians appreciate a pathologist who not only reports the lesion description and gives a morphological diagnosis, but also comments on possible differential diagnoses for those lesions and even suggests further tests that should be performed.

It is also important for laboratories to monitor trends and alert the local industry to those trends. For example, if a laboratory notes a sudden increase in submissions for air sacculitis and begins to detect a variant infectious bronchitis virus in one complex, the laboratory diagnosticians should actively pursue that agent in submissions from other complexes in the area. It could even be appropriate to alert the local industry to the occurrence of new pathogens and suggest submission of appropriate samples, while preserving the anonymity of the complexes with the actual problem. This should be a two-way street, and field clinicians should also alert the laboratory personnel when they have reason to suspect a change in a disease situation, the introduction of a new vaccine, and so forth. Searchable record systems are important. Such systems should allow internal searches by laboratory staff to identify and analyze trends. Limited outside access by field clinicians to their own data is also desirable to enable those clinicians to analyze their own data. More-or-less formal meetings between field clinicians and laboratory staff to map strategies and design projects to solve emerging problems and outbreak situations are suggested. Such meetings can be between the laboratory and a single field clinician with a single complex involved with a problem, or with multiple clinicians representing the various complexes in an area affected by a problem. Agreements can be reached on diagnostic plans of attack: what samples need to be submitted, the number of cases to examine, tests to be performed, etc.

Laboratories, especially those affiliated with universities, are often expected to function as applied research partners. This relationship is simultaneously highly important and unfortunately difficult. The modern poultry industry, by virtue of the concentration of huge numbers of birds in limited geographical areas, the prodigious growth rates of those birds, and the resulting constant and rapid turnover of populations, provides a fertile environment for the emergence of altered or new pathogens. Comparatively feeble efforts to control pathogens by biosecurity, infrequent cleaning and disinfection, and other economics-directed management decisions involving in-house densities, regional densities, down times, and so forth have led to aggressive efforts to control pathogens mainly through vaccination. This applies even more selection pressure to large pathogen populations, leading to ever more rapid emergence of variants. In less than 50 years in the US alone, we have seen the emergence of classical infectious bursal disease virus (IBD), then significantly and continually altered variant IBD viruses, Arkansas infectious bronchitis virus (IBV), Delaware 072 IBV, Georgia-98 IBV, chicken infectious anemia virus, transmissible proventriculitis, avian Metapneumovirus in turkeys, poult enteritis-mortality syndrome, "spiking mortality" (presumably infectious), and several waves of "runting-stunting syndrome", the most recent of which appears to be infectious. The "old" pathogens that we understand and for which we have vaccines are reasonably well controlled, but we are constantly faced with new situations.

When a new syndrome is detected, we want the agent identified as quickly as possible. When a new or altered agent is detected, we immediately want to know its significance and what to do about it. Is it pathogenic? Is it the proximate cause of the current problem? Will current vaccines or some combination of vaccines (especially in the case of IBV) suffice? How efficiently is it transmitted? Is it vertically transmitted? How long does it shed and persist in the environment? What other measures might be effective? It obviously is not practical to investigate each isolate of a reovirus or adenovirus or a single variant IBV for pathogenicity. However, when multiple isolations are made or trends established, rapid investigation is needed. Unfortunately, research funding is scarce and is usually dedicated to existing lists of well-identified problems. The ability to respond rapidly and forcefully to the "problem du jour" is hampered by this funding structure. A better means for industry to rapidly identify emerging short-term problems and support immediate applied research, and for academic and laboratory staff to be given the latitude, support, and especially the credit (in terms of professional advancement) to address these problems is sorely needed. Local industry and poultry federations need to be more proactive and forthcoming with monetary support, and academic and laboratory administrators need to be more flexible and supportive of such efforts.

Undoubtedly, the relationship between the field clinician, the laboratory, and the owner of the birds can be tricky. Field clinicians should not be overly demanding. The laboratory needs to work through the clinician as much as possible, without undermining the clinician, while fulfilling its professional responsibility to the owner and the birds. The directions and wishes of the field clinician need to be followed to the extent possible. Where those wishes cannot be followed, where they are clearly not indicated, or where other avenues of investigation are clearly indicated, the situation needs to be explained to the clinician in a professional manner. The laboratory clinician must fulfill all regulatory requirements while protecting client confidentiality to the extent possible.

While this paper was to focus on the field clinicians' expectations of the laboratory, the relationship should be a two-way street, and the field clinician should clearly shoulder much more than half of the responsibility for resolution of a problem. The clinician must identify the existence and urgency of the problem and communicate that to the laboratory. The clinician is responsible for proper specimen selection, collection, preservation, labeling, and transport, and legible completion of submission forms. If the field clinician is directing submission of samples by lay personnel (a very frequent occurrence) it is incumbent on that clinician to educate those laymen in proper specimen selection, collection, and so forth. The phrase "garbage in, garbage out" was never truer than in this relationship. The laboratory needs a complete, accurate history and description of signs and lesions, therapy attempted, and so on to fully meet the clinician's expectations. The laboratory expects, deserves, and depends on timely payment. Finally, the laboratory deserves professional, accurate feedback on the job it is doing.

Diagnostic Lab and Field: Overview – Perspective from the Diagnostic Laboratory:

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The diagnostic laboratory has been an integral part of my career. I have trained or worked in a diagnostic laboratory as a veterinary student, resident, poultry pathologist and presently as a poultry diagnostic laboratory director. I have also been a client of diagnostic laboratories when I worked for a primary breeding company and I am still a client when I send samples to other laboratories, so I bring to this overview my perspectives as a student, client, stakeholder and employee. For the last eleven years I have worked for the Texas Veterinary Medical Diagnostic Laboratory, a diagnostic service agency, part of the Texas A&M University System. Our laboratory system was established to provide diagnostic services to the Texas animal industries. We have two all-species laboratories (Amarillo and College Station) and two poultry laboratories (Center and Gonzales). We offer the following services: necropsy, serology, bacteriology, mycology, virology, parasitology, pathology, molecular genetics, toxicology, and field investigations. Even though we do not have research and development duties, we collaborate/interact with other agencies, departments, diagnostic laboratories and universities to provide our clients the diagnostic services they need and deserve. Our goals are to provide our clients accurate results, a rapid turn-around and affordability. Our laboratory system is accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD) and operates under a quality system that basically requires that we "say what we do" (have standard operating procedures or SOPs), "do as we say" (follow the SOPs) and document it.

A key to fulfilling the expectations of the field clinician and laboratory diagnostician is communication. Given the growth and expansion of the poultry industry, many field clinicians have responsibility for large territories and the lab diagnostician becomes the "local" veterinarian. As the local disease expert I work closely with all members of the management/production team. In order for this arrangement to work, all the players need to know and trust each other. Most of my daily interactions are with service technicians and managers. Service technicians collect most of the samples submitted to the laboratory so their level of training and experience is important. I have provided sample collection training to industry and state regulatory personnel. In my interaction with field clinicians my responsibility goes beyond providing diagnostics on a case by case basis. I keep track of emerging issues, trends and do my best to build a close relationship with the field clinicians so I can offer value not only by performing the diagnostic tests requested but also by offering suggestions. Some of my most rewarding experiences as a lab diagnostician, field clinician and research collaborator have started with a phone call or meeting. The free exchange of ideas, opinions and experiences tends to lead to a quicker resolution of complex problems. I have learned from my local, national and international experiences that the poultry industry and allied industries are like a small family. Relationships are forged under a gamut of circumstances, from casual conversations, social events, conventions and scientific meetings, to battling high consequence diseases. While on the subject of communication, a critical component of diagnostic work is the

case history. For the lab diagnostician, a case history could trigger a list of differential diagnoses and diagnostic decisions even before a single bird is examined. A good example was the rapid diagnosis and response to Avian Influenza in 2004. The birds had non-specific upper respiratory disease signs but the case history raised a red flag and Avian Influenza immediately moved to the top of the differential diagnoses, above MG, Infectious Bronchitis and others.

Another key to fulfilling the expectations of the field clinician and laboratory diagnostician is the quality and quantity of the samples submitted. As a lab diagnostician, there is no substitute for a necropsy to begin the diagnostic process as it provides the most information to determine how to proceed. The best sample is a group of birds that are representative of the problem. Symptomatic live birds are probably the best sample as they can be bled to run serological test concurrently with other test. In cases of increased mortality is very important to submit dead birds (fresh dead, refrigerated, not frozen). Submitting a "fresh" dead bird in the middle of summer is a challenge and I understand. Occasionally I have to work with a "bag of stuff" (tracheas, intestines, multiple viscera, blood samples) or fixed tissues. This is less than ideal but I am glad to work with the samples submitted as long as all involved know the limitations. Given the geography of Texas and the large territories of my clients, I understand that sometimes submitting birds is difficult. The bulk of our laboratory work is active serological surveillance (Texas Poultry Federation Program, NPIP and NCC). It is critical that we receive the number of samples necessary to fulfill the requirements of the various surveillance programs and that each sample contains enough quality serum to perform the test(s). Having plenty of serum is also important when positive test results require us to perform additional confirmatory testing.

There is no tougher test of the field clinician and laboratory diagnostician relationship than reportable or high-consequence diseases. This is when the relationships, the teams, developed during good times pay-off. Training, experience and response plans are great, but people working together are what make it work. In Texas, our Poultry Federation has over the years brought all of us to the table with the creation of a Poultry Disease Task Force and it has been the glue that has kept industry, university, laboratory, state and federal personnel working as a cohesive team. Active surveillance promoted and supported by our field clinicians, has been a key to our success.

Thanks to organizations/groups like the OIE, AAVLD, AAAP and NPIP, our laboratories follow standardized procedures that are recognized nationally and internationally. The diagnostic laboratory plays a key role in supporting the exportation of poultry products. Our laboratory participates in proficiency testing and as mentioned before has a quality system that requires the validation of procedures and the use of quality controls. The laboratory is responsible for having trained personnel and identifying non-conforming work. The quality system creates a culture of frequent communication and documentation. Continuing education is important to bring our clients the level of service they expect and is required. The growth and expansion of the poultry industry has lead to frequent inter-lab result comparisons (paired samples), but most importantly, it has lead to frequent inter-lab collaborations. As part of my job I interact and collaborate with other diagnosticians and our technical personnel do also. Our common goal is to provide our clients the best service and, with a clear understanding of our limitations, create synergies to do our best to prevent, diagnose and control poultry diseases.

Infectious Bronchitis Field Perspective: Vaccination Programs and Adjustments

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Infectious bronchitis virus (IBV) vaccination programs are as ubiquitous as IBV viruses. IBV vaccination programs should be based upon historical and /or environmental factors. IBV vaccination programs typically reference some time period of historical diagnostics, both serology and virus isolation so that IBV vaccines target known wild strain viruses. On the other hand, environmental challenges gathered by neighbors or naïve birds may be needed, especially when a new production division is introduced in an area. In short, a best guess is made for an ideal IBV program and then adjusted as flocks grow out. All IBV programs must be adjusted as vaccinated flocks demonstrate weaknesses in the IBV program.

The first step in any IBV program is picking the correct viruses and then applying the vaccines at the best possible age. However, the correct IBV vaccine virus can only work if applied correctly. All vaccine suppliers should offer technical support to ensure adequate application for successful use of their products. Vaccine suppliers interested in maintaining their business will train, audit and improve integrator vaccine administrators. Any IBV program should not fail due to inadequate administration. IBV vaccination program failure related to misapplication incriminates negligence of the vaccine supplier as much as ignorance of the customer using the vaccine.

Routine surveillance, primarily serology with ELISA kits, is the backbone to maintaining and improving IBV protection. Pullets and breeders should be monitored at critical ages during their 65 week life: pullets sometime after IBV exposure and before moving off the pullet farm, usually before administration of killed vaccines, and then as hens early in lay and again just prior to moving off the hen farm. Acute and convalescent sera still need to be collected when diagnosticians suspect IBV challenges in hens. Twenty samples per house are needed to adequately sample flocks of any size. For broilers, during good flock health, 20 sera samples from one broiler flock per week per location provide enough data to provide a baseline of IBV exposure. Vaccine virus exposure should produce some response that may be considered normal IBV "background". Every sick broiler flock may then be bled and tested when they are harvested to compare to routine flocks. In essence the baseline from healthy broiler flocks functions as acute sera and the terminal sample from the sick flock is the convalescent sera. All elevated ELISA results from pullets, hens or broilers should be typed with HI's to identify the particular IBV strain causing the high IBV titer.
Virus isolation is the gold standard of IBV flock diagnostics. Tracheas and cecal tonsils should be collected from sick flocks for virus isolation attempts. Tracheas are of most value when flocks first show clinical signs, preferably ahead of increased mortality. Cecal tonsils may be taken at any time, even from birds slaughtered and condemned at the processing plant. Training service personnel and providing a written protocol for field samples has proven necessary to obtain tracheas in a timely manner. Cecal tonsils and blood for serology may be taken just before removing the flock from the farm or at the processing plant. In order to catch samples from previously undiagnosed flocks, processing plant personnel should be instructed to at least alert grow-out personnel when processed flocks have respiratory lesions. Processing plant personnel may either obtain cecal tonsils and blood samples themselves or set aside a cage of the

affected flock for grow-out personnel to sample at a later time. Regardless of the sample, laboratories should pursue identification to the IBV serotype and not simply report "Coronavirus" as some veterinary diagnostic laboratories have done in the past 18 months. Several case studies may demonstrate how IBV programs are established and adjusted in both breeders and broilers.

Breeder IBV: An IBV breeder case resulted from studies associated with egg production drops on two particular hen farms. Two sister hen flocks, raised on the same pullet farm but moved to two hen farms several counties apart, experienced egg production drops at the same time two years in a row. The first year the egg drop was noted, blood was collected some time after the egg production returned to normal. Serum samples were analyzed for IBV, IBDV, NDV and Reo by commercial ELISA kits. The first year ELISA titer results were inclusive. "Coronavirus" was reported by one veterinary diagnostic laboratory. Another diagnostic laboratory isolated and identified IBV-Ark. IBV-Ark was expected as IBV-Ark was used in the live vaccination program. The second year, sera samples were collected immediately upon egg production drop and then again four weeks later. The acute samples were frozen and submitted with the convalescent samples. Both acute and convalescent sera were run on the same ELISA plates at the same time. The second year serology demonstrated a decline in IBV titer from historical averages at the first bleeding and then an increase closer to normal titer at the second bleeding. IBV-Ark was isolated again the second year. Timing of the egg production drop closely related to introduction of the first set of spike males. It appeared that with the live IBV vaccination program in the pullet rearing without any hen house boosting, breeder flock IBV antibody titers declined to non-protective levels. Spike males moved just 4 weeks after their last IBV vaccination which included IBV-Ark, appeared to be shedding IBV upon entry into the recipient hen house.

The solution to the breeder IBV challenge worked out to moving IBV vaccine viruses around in the pullet program more so than changing vaccine viruses. The original problematic IBV vaccination program was Mass + Conn @ 2 weeks, Mass + Ark @ 6 weeks, Mass + Conn @ 12 weeks and Mass + Ark @ 18 weeks. The final IBV vaccination program that appeared to solve the spike rooster shedding was Mass + Ark @ 2 weeks, Mass + Ark @ 6 weeks, Mass + Conn @12 weeks and Mass (Holland) + Conn @18 weeks. To date the altered program appears to have fixed the decline in egg production previously noted with the introduction of spike males.

Broiler IBV: Six years ago the corporation was enduring increased flock morbidity, mortality and processing condemnation due airsaculitis. Diagnostics repeatedly isolated IBV-Ark from affected flocks. Due to sequelae from previous vaccine virus choices, including dose and timing of administration recommended by the vaccine manufacturer, upper management would not permit IBV-Ark vaccination. IBV-Mass (Holland) was used in hopes of cross protection for IBV-Ark. However, sera and tissues were collected throughout the respiratory season and continued to point to IBV-Ark. Several farm trials were conducted to test whether hatchery alone, field alone or both hatchery and field IBV-Ark vaccination was required to protect flocks from field IBV-Ark challenge. Through the farm trials it became apparent that both hatchery and field IBV-Ark were necessary for protection. By negotiation and with much trepidation, IBV-Ark was added to the fall respiratory vaccination and IBV-Conn was removed. Both the products and dosing were different than when IBV-Ark was used for the first time. Flocks did not experience the performance problems associated with the corporation's first IBV-Ark vaccination. Since IBV-Ark has been added into the IBV vaccinations, respiratory health has

continued to improve. IBV-Del and IBV-Conn have been identified serologically with HI's, but neither has been isolated from enough flocks to warrant introduction into the broiler IBV program. In fact, this past winter season was the best respiratory health enjoyed by the corporation in over six years.

Broiler IBV new: Three new divisions have been added in the past ten years that required novel broiler vaccination programs. The goal of each new IBV vaccination program was to provide the precise IBV protection needed to maintain flock health. Without the benefit of historical diagnostics, reliance on neighbors and regional historical IBV programs provided the information to design IBV vaccination program for the new divisions.
In Bryan, TX, the only poultry neighbors were fancy bird suppliers and leghorns. Accordingly, IBV vaccinations were simply IBV-Mass+Conn for 8 years. The IBV program worked well until eggs were swapped between established complexes and Bryan. Soon after the egg swap, respiratory disease started to show up for the first time in Bryan. IBV-Ark was identified by serology and virus isolation in the sick Bryan flocks. "Neighbors" in east TX and central TX, all over 100 miles away, were not experiencing similar IBV-Ark challenges. IBV-Ark was added to the Bryan broiler IBV vaccination program and IBV-Conn was removed. Broiler flock health improved for a while and then another round of respiratory disease was noted during the next respiratory season. Most of the sick flock diagnostics pointed to IBV-Conn, despite discontinuing use of IBV-Conn vaccines for over one year. When IBV-Conn vaccine was included in the broiler IBV vaccination program, flock health returned to superior status.

In Adel, GA there were more chicken integrator neighbors than in Bryan, TX. Other broiler companies were using IBV-Mass+Conn so that became the IBV program in south GA. The IBV-Mass+Conn vaccination program was successful for the first two years of the new operation. Again eggs were exchanged between established complexes and IBV-Ark was introduced. IBV-Ark was isolated from sick flocks so IBV-Ark was added to the IBV program and IBV-Conn was removed. To date IBV-Conn has not been added back to the broiler program and flock health continues to be superior to others in the region.

The last new division added was in Waco, TX, sharing a feedmill and several farms with Bryan, TX. Due to the close proximity and interchange between Waco and Bryan, the Bryan IBV vaccination program was implemented immediately in the new Waco division. To date, almost one year after first broilers were placed, Waco broilers enjoy unmatched flock health and livability. The IBV-Mass, Conn + Ark vaccination program appears to be working well in naïve facilities as well as established farms.

From the field perspective, IBV continues to be a pathogen of much concern and cost in vaccinating, monitoring and flock illness. New technology is needed to conquer this ubiquitous pathogen. With the advent of recombinant vaccines for other pathogens it would be of great benefit to find a common immunogenic factor for IBV, so that IBV vaccines could be incorporated with the recombinant vaccines. In fact, a large part of the limiting cost of recombinant vaccines is the continued necessity for IBV live vaccinations. For the foreseeable future, conventional IBV vaccination, monitoring and adjustments will continue in commercial broiler production.

Infectious Bronchitis: Changes in the Future – Challenges from variants viruses – Can we learn from the past?

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Infectious bronchitis (IB) is among the most common and difficult of all poultry diseases to control. IB is highly contagious, has a very short incubation period and spreads rapidly via direct and indirect-airborne routes. The disease produces economic losses in commercial broiler, layer and breeder chickens. The causative coronavirus, infectious bronchitis virus (IBV), often causes respiratory disease in young chickens and egg production losses in hens. In addition, some strains of the virus exhibit a renal tropism and produce up to 30% mortality in affected flocks (22).

IBV targets the trachea and other respiratory tissues. Damage to defense mechanisms of the respiratory system which are responsible for trapping and clearing inhaled bacteria often predispose chickens to secondary bacterial infections. *E. coli* and mycoplasma may colonize the respiratory tract, causing airsacculitis, perihepatitis, and pericarditis. Chickens inoculated with IBV and *E. coli* were found to have more severe and persistent respiratory lesions than those inoculated with IBV alone (13). Other factors such as ammonia, temperature fluctuations, and social stress (pecking) contribute to the susceptibility of layer type chickens to bacterial infections (17). In addition, immuno-suppressive infections with IBDV in combination with IBV, reduced macrophage activity vs. *E. coli* (14) and increased IBV persistence (16, 18).

IBV is well known for its existence as numerous antigenic types or serotypes. Antigenic viral variation has been recognized since the 1950s. Today, genetic analysis is used to compare differences among strains. A NCBI (http://www.ncbi.nlm.nih.gov/) database search for "IBV" revealed 1664 sequence results of which 799 were for the spike (S) glycoprotein gene. The S protein, used by IBV for attachment to the host cell, displays extensive genetic and antigenic variation. Strains differing greatly in the hypervariable regions of the S gene compared to vaccine strains have the potential to break through vaccinal immunity.

Other genes of the virus, although less well studied, undoubtedly contribute to the biology of IBV-pathogenicity, immunogenicity, and tissue tropism. However, new research emphasizes the importance of looking beyond the S gene to understand the complexities of the virus. Mardani and coworkers (12), retrospectively comparing strains from chickens in Australia, reported the discovery of IBVs with novel genome organizations. Unlike the "classical" IBV gene organization, the "novel" genomes lacked genes (3a, 3b, 5a, 5b) coding for nonstructural proteins at the 3' end of the genome (see below). Moreover, the "novel" genomes had an additional gene, X1, which had no homology to any other IBV gene. The gene order for the novel strains was either:

The "classical" genome organization for IBV, a group III coronavirus.

5'-Pol-S-3a-3b-E-M-5a-5b-N-untranslated region (UTR)-3'

The "novel", newly discovered genome organizations for IBV

5'-Pol-S-X1-E-M-N-UTR-3'
5'-Pol-S-X1-E-M-5b-N-UTR-3'

Phenotypically, the novel and classical IBVs also differed; the novel IBVs grew more slowly and reached lower titers *in vitro* and *in vivo*, were of lower immunogenic in chicks, and displayed different tissue tropism-respiratory vs. renal. These recent findings indicate that the diversity among IBV is greater than previously thought.

Variant Selection is Influenced by Production Practices

New variant IBV strains are subject to immunological selection so only the most antigenically novel variants persist in poultry populations. A new variant that is not antigenically novel, e.g. one similar to a vaccine strain used on a farm, will not persist in the flock because the vaccine-induced immunity will eliminate it from the population. Conversely, newly mutated variants that are antigenically distinct from vaccine strains will, in essence, have a far greater potential to escape vaccine-induced immunity, persist in flocks, and have the potential to cause disease.

The primary source of highly novel IBV variants are commercial layer flocks. The incidence of variants in commercial layers, especially those raised in multiple age flock operations is high, much higher than in broiler flocks. Layers frequently numbering in excess of a million birds, of different ages and IBV immune status, are housed in close proximity, facilitating rapid transmission. Periodic introduction of new pullets, and the continual re-infection and recycling of IBV in the layers, results in a greater opportunity for infection and spread than occurs on farms using a single age flock, "all in-all out" management system. Novel IBV variants build up in layer complex houses over time. Importantly, vaccine induced immunological mechanisms provide a selective pressure for the most antigenically novel variants as new variants arise on a frequent basis. Variants that evolve in layer flocks pose a biosecurity threat to other farms nearby.

Another important and common source of pathogenic IBV is live attenuated vaccines that have undergone reversion to virulence under field conditions (15). This problem is often seen in broiler production. Attenuated vaccines contain millions of highly related but not identical virus particles referred to as quasispecies or subpopulations. The subpopulations in the vaccines differ in their virulence (level of attenuation) and even slightly in their antigenic characteristics representing subtypes of the virus. Live IBV vaccines contain mixed subpopulations, a characteristic that makes them "unstable" and capable of reversion to virulence depending on the host (embryonated egg vs. chicken-natural host) in which they are grown. For example, back-passage of embryonated egg-derived vaccine in the chicken results in shifting of the viral subpopulations in favor of a predominant virulent subpopulation over the attenuated subpopulation found in the original vaccine. Similarly, a slight shift or change in the antigenic characteristics of the vaccine may also occur through back-passage. Overtime, and in the absence

of farm clean out and disinfection, back-passaged vaccine may become established as a significant IBV challenge. Indeed, laboratory studies have shown that vaccines may revert to partial or full virulence within only 3-6 back-passages in chickens (5). Practices associated with enhancing vaccine back-passage should be limited. These include over-diluting (cutting) IBV attenuated vaccines, using sprayers that apply wide-ranging particle sizes, and using a vaccine strain(s) only on a seasonal (winter only) basis. Using quantitative RT-PCR, Jackwood and co-workers (6) reported poor distribution of IBV vaccine when given by spray. Pathogenic IBV vaccine-derived field isolates are capable of causing disease in broilers (15,20).

Current Variant Status

California. A variant, CA/1737/04, has continued to be isolated from broilers since 2004, and more recently from 4-6 week old pullets, the latter showing nephritis (7). From 2003-2007, a relative increase in the isolation of Conn genotype isolates was noted. Monoclonal antibody testing and multiple gene analysis revealed differences among the isolates. Characteristics of the Conn isolates suggest that they are of vaccine origin in most cases although recombination has also been observed in one isolate (21).

Connecticut. No activity reported (M. Khan, personal comm.)

Delmarva. A "new" variant, DE/5642/06, was recovered from four broiler flocks with respiratory disease in February 2006, but not thereafter (2). Vaccinal genotypes continue to be isolated in high numbers from broilers with respiratory disease. Arkansas IBV isolations represent the vast majority of the genotypes recovered (University of Delaware Poultry Health System).

Georgia. New variants, GA07 and GA08, were reported in 2007 and 2008, respectively. GA07 viruses were isolated from broilers or broiler breeders with nephropathogenic IB. GA08 was recovered from broilers with acute-chronic respiratory disease (airsacculitis) and colibacillosis. S1 sequencing suggests these variants to be novel (18).

North Carolina. Arkansas IBV reported (G. Erickson, personal comm.)

Texas. Variants have not been reported. A few vaccinal serotypes (Connecticut, Delaware, and Arkansas) have been recovered from broilers with chicken anemia or infectious bursal disease. (J. Linares, personal comm.).

Diagnosis of IB

Serologic Detection. ELISA is a preferred method for detecting serum antibody response, but it does not identify serotype-specific antibodies. Acute and convalescent sera responses are useful in diagnosis. Antibody responses to vaccination generally produce lower ELISA titers than is observed following a virulent field challenge.

Hemagglutination-inhibition (HI) tests may be performed on sera from commercial broilers to identify a causative serotype responsible for IBV infection. However, HI antibodies produced in response to multiple IBV vaccinations and field exposures can become cross-reactive and non-specific and thus may not be indicative of the strains responsible for recent disease episodes (1).

Antibodies produced by young chickens are more serotype-specific, whereas cross-reactions with heterologous VN and HI antigens are common in breeders and layers.

Virus isolation and identification. Virus isolation may be accomplished directly from diseased broilers or through sentinel studies (3). Trachea or lung is preferred. The cecal tonsil is often persistently infected with IBV vaccine and field strains as well as other viruses, thus making interpretation of IBV isolation from cecal tonsil difficult. RT-PCR assays for IBV have been developed (8,9,10). Sequence analysis of the S1 gene is the preferred method for identification (11). The major uses of RT-PCR tests are virus identification and its application in the understanding of epidemiological investigations during IBV outbreaks. The RT-PCR tests, as they now exist however, do not provide information on viral pathogenicity.

Control of IB by Vaccination

Available live strains. Selection of appropriate IBV vaccine serotypes is essential to achieve the best protection against endemic field strains. In the U.S., live vaccines are available for four serotypes; Mass, Conn, Ark and Delaware 072/GA 98. Vaccines are routinely given in combination with a vaccine strain of lentogenic Newcastle disease virus. IBV vaccine serotypes are generally given as monovalent or bivalent combinations.

Cross-protection. Some strains used alone or in combination with other of strains (Mass and Ark protect against many strains) (4) induce cross-protection that has practical benefits. However, there is no way of predicting the level, if any, of protection vs. challenge with a variant strain. Laboratory vaccination-challenge of immunity studies must be performed determine if cross-protection will be realized.

Vaccination with multiple (3 or more) strains. There are limits of the ability of the chicken to respond to multiple simultaneously-applied IBV modified live vaccines. Immunity is the outcome of the more reactive (virulent) strains contained in the vaccine. For example, bivalent vaccination (two live strains given simultaneously) will produce immune responses to each of the two strains. However, simultaneous application of three IBV strains may not produce the desired results. Studies using Mass, Conn, and JMK live vaccines applied via eyedrop produced immunity to two of the challenge viruses (Mass and JMK), but little immunity was produced against the third challenge virus (Conn) (Gelb, unpublished).

Inactivated vaccines. Inactivated vaccines require injection of individual birds. Inactivated vaccines are generally administered between 10 and 18 weeks of age and are given 2–4 weeks after a series of 3–4 priming immunizations with live vaccine. They are often given in combination with other inactivated vaccines. The efficacy of inactivated vaccines given by respiratory routes to broilers is debatable. Although spray application of inactivated IBV is attractive in terms of inducing immunity in the respiratory tract without side effects associated with live vaccines, this approach has not demonstrated sufficient efficacy. Vaccine cost is also a consideration.

Future

Many more IBV variants will be discovered, which will pose challenges to the poultry industry and vaccine developers. Since variants in many cases are not geographically widespread, there will be little incentive to produce new vaccines. The following are areas to consider.

Biosecurity
Challenge reduction
Evaluate cross-protection potential of current vaccines
Recognize and reduce impact of immunosuppression, where feasible.
Develop "safer" modified live virus vaccines-reduced transmissibility and persistence
Evaluate other vaccine approaches-recombinant and inactivated possibilities

References

1. Gelb, J. Jr. and S. L. Killian. Serum antibody responses of chickens following sequential inoculations with infectious bronchitis virus serotypes. Avian Dis. 31:513-522. 1987.

2. Gelb, J., Jr., B. S. Ladman, C. Pope, and M. Wood. Characteristics of novel infectious bronchitis virus isolates from Delmarva broiler chickens. Proc. 144th American Veterinary Medical Assn./American Assn. Avian Pathologist Ann. Mtg. Washington, D.C. July 14-18, 2007.

3. Gelb, J., Jr., J. K. Rosenberger, P. A. Fries, S. S. Cloud, E. M. Odor, J. E. Dohms, and J. S. Jaeger. Protection afforded infectious bronchitis virus-vaccinated sentinel chickens raised in a commercial environment. Avian Dis. 33:764-769. 1989.

4. Gelb, J., Jr., J. B. Wolff and C. A. Moran. Variant serotypes of infectious bronchitis virus isolated from commercial layer and broiler chickens. Avian Dis. 35:82-87. 1991.

5. Hopkins, S. R. and H. W. Yoder, Jr. Increased incidence of airsacculitis in broilers infected with *Mycoplasma synoviae* and chicken-passaged infectious bronchitis vaccine virus. Avian Dis. 28:386-396. 1984.

6. Jackwood, M. W., D. A. Hilt, and E. T. McKinley. The dynamics of spray vaccination for IBV in commercial broilers. Proc. 144th American Veterinary Medical Assn./American Assn. Avian Pathologist Ann. Mtg. Washington, D.C. July 14-18, 2007.

7. *Jackwood M. W., D. A. Hilt, S. M. Williams, P. Woolcock, C. Cardona, and R. O'Connor. Molecular and serologic characterization, pathogenicity, and protection studies with infectious bronchitis virus field isolates from California. Avian Dis. 51: 527–533. 2007.*

8. Keeler, C. L., Jr., K. L. Reed, W. A. Nix and J. Gelb, Jr. Serotype identification of avian infectious bronchitis virus by RT-PCR of the peplomer (S-1) gene. Avian Dis. 42:275-284. 1998.

9. Kingham, B. F., C. L. Keeler, Jr., W. A. Nix, B. S. Ladman, and J. Gelb, Jr. Identification of avian infectious bronchitis virus by direct automated cycle sequencing of the S-1 gene. Avian Dis. 44:325-335. 2000.

10. Kwon, H. M., M. W. Jackwood, and J. Gelb, Jr. Differentiation of infectious bronchitis virus serotypes using the polymerase chain reaction and restriction fragment length polymorphism analysis. Avian Dis. 37:194-202. 1993.

11. Ladman, B. S., A. B. Loupos, and J. Gelb, Jr. Infectious bronchitis virus S1 gene sequence comparison is a better predictor of challenge of immunity in chickens than serotyping by virus neutralization. Avian Path. 35: 127-133. 2006.

12. Mardani, K., A. H. Noormohammadi, P. Hooper, J. Ignjatovic, and G. F. Browning. Infectious bronchitis viruses with a novel genomic organization. J. Virol. 82: 2013-2024. 2008.

13. Nakamura, K., J. K. A. Cook, J. A. Frazier, and M. Narita. Escherichia coli multiplication and lesions in the respiratory tract of chickens inoculated with infectious bronchitis virus and/or E. coli. Avian Dis. 36:881-890. 1992.

14. Naqi, S., G. Thompson, B. Bauman, and H. Mohammed. The exacerbating effect of infectious bronchitis virus infection on the infectious bursal disease virus-induced suppression of opsonization by *Escherichia coli* antibody in chickens. Avian Dis. 45:52-60. 2001.

15. Nix, W. A., D. S. Troeber, B. F. Kingham, C. L. Keeler, Jr. and J. Gelb, Jr. Emergence of subtype strains of the Arkansas serotype of infectious bronchitis virus in Delmarva broiler chickens. Avian Dis. 44:568-581. 2000.

16. Pejkovski, C., F. G. Davelaar, and B. Kouwenhoven. Immunosuppressive effect of infectious bursal disease virus on vaccination against infectious bronchitis. Avian Path. 8: 95-106. 1979.

17. Phillip, H. C., and M. Voss. What do we know about coli infections in commercial layers. Lohmann Information. No. 25. Pp. 1-3. Lohmann Animal Health. http://www.lah.de/ 2001.

18. Rosenberger, J. K. and J. Gelb, Jr. Response to several avian respiratory viruses as affected by infectious bursal disease virus. Avian Dis. 22:95-105. 1978.

19. Sellers, H. S., B. E. Telg, and S. M. Williams. Recent isolation and characterization of nephrotropic and variant infectious bronchitis virus isolates from Georgia. The Poultry Informed Professional®. University of Georgia. S. Collett and P. Villegas, eds., issue 98. March/April 2008.

20. Van Santen, V. L., H. Toro, K. S. Joiner. Rapid selection in chickens of a subpopulation within an attenuated infectious bronchitis virus vaccine. Proc. 144th American Veterinary Medical Assn./American Assn. Avian Pathologist Ann. Mtg. Washington, D.C. July 14-18, 2007.

21. Woolcock, P. R. and C. J. Cardona. Infectious bronchitis viruses isolated in California 2004-2006. Proc. 144th American Veterinary Medical Assn./American Assn. Avian Pathologist Ann. Mtg. Washington, D.C. July 14-18, 2007.

22. Ziegler, A. F., B. S. Ladman, P. A. Dunn, A. Schneider, S. Davison, P. G. Miller, H. Lu, D. Weinstock, M. Salem, R. J. Eckroade, and J. Gelb, Jr. Nephropathogenic infectious bronchitis in Pennsylvania chickens 1997-2000. Avian Dis. 46:847-858. 2002.

Use of vaccines in the control of ILT outbreaks in broilers: Vaccination in the face of ILT infection

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Introduction. The control of infectious laryngotracheitis (ILT) requires a multidisciplinary approach involving at least the application of epizootiological studies, biosecurity, cleaning and disinfection, judicious management of contaminated litter and equipment, and controlled live hauling of infected flocks (2, 5). An additional common recourse for the control of ILT is vaccination whether it is used preventively or "therapeutically". Although apparently counterintuitive, abstinence from vaccinating susceptible flocks with live attenuated vaccines as part of the control programs for ILT is an appropriate approach in certain circumstances.

Available vaccines and their general characteristics. The first vaccines made available to the poultry industry in the first part of the 20th century were represented by poorly attenuated infectious laryngotracheitis virus (ILTV) (3, 10, 15, 16). Although protective, such vaccines typically induced reactions as severe as contemporary field viruses. Until recently, the only commercial vaccines available to the poultry industry involved viruses propagated and attenuated in cell culture and/or in chicken embryos, hence the acronyms TCO (tissue culture origin) (8), and CEO (chicken embryo origin) (9). The "Cover" and "Hudson" strains of ILTV represent the two most commonly used vaccine strains for the protection against ILT. CEO vaccines may be delivered by mass application methods such as spray or via the drinking water, and also by individual bird application methods such as ocular, intranasal or intracloacal. CEO vaccines may be given only by individual bird application methods. CEO vaccines provide perhaps the best and longest flock protection, albeit they spread more easily, can back passage while gaining virulence and can cause severe vaccine reactions or even vaccinal LT (12, 13), commonly known as "VLT". Circumstantial field observations have suggested that the simple use of CEO vaccines in broilers may result in delayed growth and increased feed or caloric conversion, both being undesired effects derived from the need of vaccinating broilers in the face of an outbreak. TCO vaccines are known to be less reactive and less prone to spreading laterally than CEO vaccines, and they provide adequate flock protection (12). However, TCO vaccines can only be applied by individual bird application methods if adequate protection is to be attained.

At least two recombinant ILT vaccines have been in use in the field recently and more will be made available in the coming months. The vaccines already in use include a Fowlpox-vectored, and a Herpesvirus of Turkeys- (HVT) vectored ILT vaccines. Both vaccines are recommended and labeled for use after hatch. However, because such vaccines can only be given by injection and since the broiler industry no longer vaccinates broiler chicks by injection post-hatch, both types of vaccines have been used extensively off-label for *in ovo* vaccinations. Recombinant vaccines are still being evaluated in terms of actual protection and duration of immunity. It is important also to explore possible factors interfering with protection conferred by recombinant vaccines. For example, possible interference of maternal antibodies with the

Fowlpox or HVT vectors may reduce the efficacy of the vaccines at least partially. The use of other non-recombinant vaccines to protect flocks against Marek's disease and Fowlpox may also interfere with the efficacy of the recombinant vaccines. In addition, the timing of in ovo vaccination appears to be a significant factor influencing protection. Finally, the potential for Fowlpox vaccines to induce pathological changes in embryos or young chicks when given in ovo remains controversial and should be investigated.

Methods of vaccination. Although several types of vaccines may be given by multiple possible routes of application, the only methods that can be used practically in broilers include *in ovo* vaccination with recombinant vaccines in the hatchery, and/or vaccination via the drinking water or by spray in the field. Vaccination in the hatchery is typically done at 18.5-19 days of incubation (444-456 hours of incubation). Vaccination at an earlier embryonic age may result in poor protection against Marek's disease or ILT, and even in pathological changes associated with Fowlpox virus. The use of live attenuated vaccines in the field requires administration after 10 days, since broilers are not fully receptive to the virus prior to this age, a phenomenon thought to be not related to maternal antibody (1, 4, 6, 7). Although ILT vaccines may be given at any age after 10 days, circumstantial evidence has indicated that vaccine reactions become harsher as broilers age, and thus the age of vaccination should not be delayed beyond 21 days of age when possible. ILT vaccines induce very little and manageable reaction when given before 10 days of age and thus it would seem that ILT vaccinations should be done before 10 days of age, but the protection attained is unsatisfactory. Amongst the mass application methods used in the field, spray application is the fastest, most practical and possibly the one that provides the best protection, but it is often the one inducing the harshest reactions, the most economic detriment, and the one associated with VLT in the field (11-13). Water vaccinations, when properly done, are less prone to deriving in all the disadvantages that have been attributed to spray vaccinations. Under typical commercial settings, it takes 2-3 personnel approximately 5 minutes to vaccinate 22-28,000 broiler chickens by spray, whereas it may take the same personnel 45-90 minutes to vaccinate the number of broilers. It is for this reason that many broiler integrators refuse to use water vaccination methods. There are various approaches for water vaccination, but the two most common ones involve the use of medicators that deliver one or more ounces of vaccine for every gallon of drinking water, and the method of water-pump vaccination. The use of medicators is unsuitable, because it takes too long for the vaccine to be delivered in full to the drinker lines, which results in vaccine virus titer decrease and it forces personnel to remain with the birds too long stimulating them to drink the vaccine. Water-pump vaccination is usually a satisfactory method because the use of immersion water pumps forces the vaccine rapidly into the water lines thus avoiding significant decrease in virus titer. If properly water-deprived, most broiler flocks should consume the vaccine within one hour and the vaccine will not sit in the drinking water lines any significant amount of time.

Concomitant vaccinations. ILT-affected areas are typically challenged with multiple other pathogens. Thus, it is not uncommon for broiler flocks to have to be vaccinated also against Newcastle disease virus (NDV) and infectious bronchitis virus (IBV). Virtually all broilers are vaccinated against NDV and IBV in the hatchery, and most are also vaccinated in the field. During outbreaks of ILT or VLT, many integrators vaccinate against ILT along with NDV and/or one or more serotypes of IBV. The broiler integrators with the least amount of vaccination-associated problems are those that vaccinate against ILT while minimizing vaccinations against other respiratory viruses. Recent field experience has revealed that VLT

and respiratory disease in general tend to be a more significant problem in operations that spray-vaccinate broilers with ILT vaccines mixed with other respiratory viruses, particularly if spray vaccinations are done after 18-21 days of age.

Legal and commercial considerations. It is important to consider that in many instances, vaccination with live viruses is illegal within 21 days prior to slaughter. Thus it is probably impractical and possibly unprofitable to vaccinate broilers that are to be slaughtered before 35-40 days of age. In addition, vaccination of chickens with CEO vaccines is illegal in some areas or may require the approval of the State Veterinarian. Finally, some importing countries may require that the imported product is not from ILT-positive areas or from vaccinated flocks.

Vaccinating broilers in the face of an outbreak. As stated before, the control of infectious laryngotracheitis (ILT) requires a multidisciplinary approach involving at least the application of epizootiological studies, biosecurity, cleaning and disinfection, judicious management of contaminated litter and equipment, and controlled live hauling of infected flocks (5). The poultry industry has learned that ILT or VLT are better controlled by using coordinated efforts by all poultry companies in an affected area (2, 5). The use of geographic information systems (GIS) that physically localize poultry farms, processing plants and live haul routes in affected areas has been helpful to rapidly determine "vaccination zones", live haul routes, biosecurity zones and zones of restricted transportation and spread of contaminated litter. The aim should be to vaccinate broilers exclusively where prescribed within a specific vaccination zone and within a specific period of time. That is, all companies represented within the vaccination zone must vaccinate birds of proper age for as long as the prescribed vaccination period lasts, and all vaccination in broilers should stop once the outbreak is declared controlled and no more outbreaks are detected. Detailed guidelines have been provided elsewhere as to recommended biosecurity, cleaning, disinfection, litter management and transportation of chickens from affected areas (2, 5). It is important to consider that the control of ILT involves much more than simple vaccinations. Regarding broiler vaccinations, recent field experiences have produced the following important observations:

a. **Industry coordinated vaccination.** Leaving unvaccinated naïve flocks in an outbreak zone where vaccination is practiced provides an opportunity for vaccine viruses to be back passaged, regain virulence, and cause VLT problems. Thus, all broiler integrators must participate with vaccinating their flocks within a defined outbreak zone. A suggested concerted industry approach for ILT control has been detailed elsewhere (5). Briefly, the only plausible avenue for accomplishing a coordinated vaccination program for broilers in the face of an outbreak is by establishing a communication system between all integrations in the affected area and by all companies in the area agreeing to initiate and stop vaccination in a coordinated fashion. It is also critical to define clearly vaccination zones and to establish specific biosecurity zones and procedures. The establishment and modification of any vaccination zones relies heavily on the assistance provided by geographic information systems (GIS), which are critical for designing vaccination and biosecurity zones. Live transportation of broilers to slaughter must follow routes with the least possible exposure to susceptible flocks. A contaminated litter management program must be defined and enforced. Downtime in affected houses should be increased as much as possible and it should not be less than 14 days, but ideally 21 days or more. Finally,

contaminated litter should be heat-treated and/or composted. Although some of these procedures are not directly related to vaccination, it is important to incorporate them into any ILT control program for broilers, since vaccination alone is unlikely to be effective in controlling the disease. Deciding when to start vaccinating an affected area is not as difficult as deciding when and how to stop vaccinations. Vaccination is usually started after 2 or more cases that are geographically and chronologically close to each other are recorded. Vaccination is usually adopted if many neighboring flocks are considered "at risk". Vaccination is usually stopped in all integrations in the vaccination zone after no outbreaks or cases are observed in at least 2 broiler grow outs. Although this process would seem simple, one must keep in mind that when vaccination actually stops there will still be many vaccinated flocks potentially shedding virus in the area and placing at risk the flocks that will not be vaccinated in the zone. This concept applies for any vaccination zone where live attenuated vaccines are used. A concerted industry vaccination approach is most often the best avenue, albeit in some circumstances, abstinence from vaccination with live attenuated vaccines may be a suitable approach.

b. **Water-pump vaccination vs. spray vaccinations.** Although many broiler integrations are successful with spray vaccinations and experience few complications, water-pump vaccinations are considerably less prone to inducing VLT. Spray vaccinations often result in VLT, at least in a small proportion of vaccinated flocks.
c. **Concomitant vaccinations.** In many cases it is unavoidable to have to vaccinate broilers against respiratory diseases other than ILT. However, every effort should be made to limit the number of vaccinations, especially if they must be given along with ILT vaccines. Recent field experience has suggested that the broiler integrations with more significant complications were those that used spray vaccinations to immunize flocks against ILTV, especially when multiple other viruses were used in conjunction with ILT vaccines.
d. **Age of vaccination.** Age of vaccination does not determine whether flocks will experience VLT or not. However, more flocks are likely to experience VLT problems if vaccinated "late", meaning after 18-21 days of age. This does not necessarily imply that flocks vaccinated after 21 days will experience VLT problems. However, integrations that vaccinate "late" against VLT or ILT with CEO vaccines are more likely to experience a higher frequency of VLT cases or complications than integrations that vaccinate properly a younger ages. In addition, most recorded cases of ILT or VLT diagnosed in the laboratory tend to occur at or later than 21 days of age, but most commonly at or later than 35 days of age (2).
e. **Flocks of different economic function or purpose.** Many poultry producing areas experiencing VLT or ILT do so because they contain multiple types of commercial poultry in close proximity. For instance, regions in the South Eastern United States may house in relatively close proximity broilers, broiler breeders, grandparent and great grandparent flocks, multiplier flocks, commercial layers, layer breeders and quail in a relatively small geographic area. In addition, non-commercial or non-traditional poultry populations and their traffic may be challenging to deal with since they may act as a reservoir for ILTV and they are not typically vaccinated against ILTV. In areas where CEO vaccines are approved, commercial layers are commonly vaccinated against ILT or VLT with CEO vaccines, while meat type breeding stock tends to be vaccinated with TCO vaccines and/or recombinant vaccines. Game fowl

reared in the same area are not typically vaccinated against ILT and may also act as a reservoir of ILTV. Finally, a significant number of birds are bred and grown for non-traditional markets or purposes including (not exclusively) poultry shows, flea markets, auctions, etc. Such variety of approaches and types of poultry certainly make it more difficult to implement successful methodologies by the poultry industry to control VLT or ILT.

f. **Types of vaccines and their field performance.** As stated above, the only types of vaccines that can be used practically in broilers include vaccines that can be applied by mass application methods. For this reason, CEO vaccines are the only kind of live attenuated vaccines used in the field. Recombinant vaccines have been used extensively off-label for *in ovo* injections of both Fowlpox- and HVT-vectored vaccines. Over the years, field use of CEO vaccines has produced overall good protection albeit with inconsistent results and frequent complications resulting in VLT or related problems (11-13). Recent field experience with recombinant vaccines has produced controversial results. Broiler integrations producing relatively small and young broilers (less than 42 days of age) have opted for using recombinant vaccines *in ovo*, while integrators producing a "mid-size" to "large" broiler (49-65 days) have chosen to vaccinate them with CEO products. Initial experiences with recombinant vaccines in "large" broilers were suboptimal in the face of overwhelming field challenge. However, recombinant vaccines appear to have performed well in broilers slaughtered at ages younger than 45 days. Although mortality and clinical signs can be noticeable in flocks vaccinated with recombinant vaccines, they are substantially less significant than in non-vaccinated flocks or than in flocks with clinical VLT. Although much has to be learned still regarding recombinant vaccines through controlled studies, it would appear that a suitable control program in the face of a serious outbreak could involve recombinant vaccinations for small and mid-sized broilers; and CEO water-pump vaccinations for large broilers with or without recombinant vaccinations *in ovo*.

g. **The role of recombinant vaccines in the overall control of VLT and ILT.** Based on field experience, it is possible that recombinant vaccines alone may not withstand an overwhelming field challenge as well as mass-applied CEO vaccines or individually-applied TCO vaccines would, but they still have the advantage of protecting flocks at least partially without seeding large geographical areas with viable virus like CEO vaccines may do in the face of a serious outbreak. Recombinant vaccines present the possibility of providing some level of protection without the disadvantage of replicating virus seeding farms and back passaging to produce VLT at least in some flocks. It is possible that the use of CEO vaccines in young broilers intended for slaughter at of before 45 days of age may actually aggravate some situations. For example, recent research has demonstrated that vaccine viruses are still detectable up to 21 days post-vaccination in the tracheas of vaccinated or contact chickens (14). Thus, if a flock of young broilers is to be vaccinated at approximately 14-21 days of age, such flock could potentially shed a significant amount of virus until at least 35-42 days of age or more. This means that vast numbers (millions) of vaccinated broilers would be transported to processing plants while still shedding CEO vaccine virus, whereas "large" (heavy) broilers slaughtered at 55-65 days of age are sent to slaughter when they are no longer shedding virus and thus they do not pose a significant risk like young broiler flocks

do when they are vaccinated with CEO vaccines. For these reasons a successful approach for control of infectious laryngotracheitis in the face of an outbreak could involve vaccination of "small" broilers with recombinant vaccines only, and vaccination of "large" broilers with CEO vaccines alone or in combination with recombinant vaccines.

Therapeutic or emergency vaccination. By definition, all vaccinations serve to prevent flocks against the clinical and economic effects derived from field infection should it occur. However, given the fact that ILTV spreads relatively slowly within broiler houses, between houses in a farm and sometimes between farms, it is possible in some circumstances to vaccinate flocks "therapeutically". In reality, it is not possible to vaccinate therapeutically against ILTV. A more appropriate term could be "emergency vaccination" against ILTV. Such emergency vaccination is routinely practiced in broiler farms of other countries where the cost of labor is more accessible and where individual bird vaccination by eye drop can be done before an outbreak of ILT spreads through an entire farm. A similar approach is followed in layer and breeder farms in the United States, where ILT vaccines may be given rapidly and successfully in the water as an emergency measure after one or more houses in a given farm or complex are confirmed as being affected by a clinical case of ILT or VLT.

In summary, a multidisciplinary approach is required to control ILT. Such approach includes the use of vaccines, but one must always keep in mind that vaccination alone will rarely be sufficient to control ILT or VLT in broilers, particularly in densely populated poultry producing areas, and more so where different types of poultry converge in the same geographic area. There are instances when abstinence from vaccinating susceptible flocks with live attenuated vaccines may be a suitable approach.

Infectious Laryngotracheitis (ILT)

Diagnostics Results: Interpreting PCR, Histopathology, and Virus Isolation

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Infectious laryngotracheitis (ILT) is an upper-respiratory disease of poultry with a worldwide distribution. The disease is common in areas of intense poultry production and during severe outbreaks it causes great economic losses due to moderate mortality, moderate to severe morbidity, and reduction of egg production. Presumptive diagnosis of ILT can be made reliably in cases of severe acute disease based on increased mortality with typical signs of the disease such as expectoration of blood. Otherwise, diagnosis should be based on one or more confirmatory laboratory diagnostic procedures. However, even if the classical clinical signs for ILTV are presented, many other respiratory diseases can cause similar signs, therefore a clinical diagnosis of the disease is not reliable. Among other infections that can appear clinically similar to ILTV are the diphtheritic form of avian poxvirus and infections caused by Newcastle disease virus, avian influenza virus, infectious bronchitis virus, fowl adenovirus, mycoplamosis and *Aspergillus* spp (13).

The most common rapid laboratory diagnostic procedures for ILT are histology, confirmed by virus isolation, detection of ILTV antigen in tissues, and/or detection of ILTV-specific DNA (13). Histopathology examination remains the standard method for the rapid diagnosis of ILTV. Histopathological lesions characteristic of ILTV, include syncytial cell formation with eosinophilic intranuclear inclusion bodies (INIB), necrosis, and hemorrhage of the conjunctival and tracheal epithelial cells. Inclusion bodies are present only during the early stages of infection (1 to 7 days post-infection), during the peak of clinical signs, and disappear as infection progresses as a result of the necrosis and desquamation of the epithelium (24). If ILTV is suspected but histopathological examination of hematoxylin and eosin stained tissues yields tentative results, not obvious INIB, lack of syncytia formation, or only degenerated and ulcerated epithelial cells are observed, immunohistochemistry (IHC) with staining of formalin fixed tissues has been very useful for the detection of the viral antigen in association with the tracheal and conjunctiva lesions (13). During mild forms of the disease, the histopathological diagnosis can be difficult because infrequent number of intranuclear inclusions with or without syncytial cell formation are commonly observed (24). In those cases it was a successful strategy to initially perform several passages in chorioallantoic membrane (CAM) of embryonated eggs and them perform histopathological and IHC analysis of the CAMs (24). In addition to histopathology other rapid assays such as the use of direct fluorescent-labeled polyclonal antibodies (FA) to detect viral antigens in tracheal and conjunctival smears (12), or in frozen tracheal sections (7, 14) have been successfully utilized in the rapid diagnosis of the disease.

Virus can be isolated from trachea, conjunctiva, lungs homogenates in specific-pathogen-free (SPF) chicken embryos inoculated via the CAM route followed by the identification of

plaque formation characteristic of ILT viral replication. Virus isolation is also possible in primary chicken embryo kidney (CEK), chicken embryo liver (CELi), or in chicken kidney (CK) cells. Viral isolation may take three or more passages before plaque formation or cytopathic effect caused by ILT replication appears in the CAM or primary chicken cell cultures, respectively. In addition, ILT replication in primary chicken cell cultures is easily overgrown by other viruses that will mask the cytopathic effect of ILT (17). Most laboratories do not rely merely in the cytopathic effect caused by ILT as a final diagnosis and perform additional tests to confirm the isolation results. Confirmatory tests included histopathology examination, PCR, or FA on the CAM or tissue culture material.

In the past decade, conventional polymerase chain reaction (PCR)-based assays in combination with histopathology examination have been utilized for the rapid diagnosis of ILTV in poultry. Conventional PCR-based assays have been used to successfully detect ILTV DNA from the trachea of experimentally (1) and naturally infected chickens (19, 28), and from extra-tracheal sites such as the conjunctiva (2) and the trigeminal ganglia (16, 28). In addition, conventional PCR has proven to be useful to detect ILTV infected birds both during severe (28) and mild epidemics (24) of the disease.

With the advent of the polymerase chain reaction, restriction fragment length polymorphism of PCR products (PCR-RFLP) has greatly facilitated the differentiation of ILTV strains. PCR-RFLP and sequencing analysis of single and multiple viral genes and genome regions has permitted the differentiation of ILTV isolates from vaccine strains in different parts of the world (5, 6, 11, 15, 16, 19). The use of multiple gene sequence analysis and multiple PCR-RFLP has been essential to identify informative single nucleotide polymorphism (SNP) appropriate for the discrimination of isolates from a particular region (20, 21, 22). PCR-RFLP analysis of informative SNPs has been utilized to differentiate field isolates form vaccine strains (6). However, differentiation of strains based in one or multiple regions of the genome needs to take in consideration the previous strains circulating in the regions as well as the vaccine types utilized. Consequently, PCR-RFLP assays need to be tailored for each country in order to precisely characterize the ILTV isolates circulating in a particular region at different time points.

Most recently the development of a real-time (ReTi) PCR assay using a Taqman® labeled probe for the detection and quantitation of infectious larygotracheitis virus (ILTV) in chickens has been reported (4). This assay has been utilized to evaluate field vaccination dynamics of live-attenuated ILTV vaccines (10) and as diagnostic tool in the field (7). As a diagnostic tool Real-time PCR provided valuable information about the amount of virus present in the diagnostic samples. Samples with genome copy numbers (GCN) greater than 4 $\log_{10}$ were observed in cases where histology and FA were positive, while in cases were the viral nucleic acid concentration was lower than 4 $\log_{10}$ the histology examination and FA assays were negative and clinical disease was not as apparent. These results may be interpreted as false-positive cases, as birds that where expose to the virus but the viral load was not sufficient enough to cause a complete clinical manifestation of the disease, or birds that were sampled during the chronic stages of the disease were the virus replication in the upper respiratory tract slows down and the birds become carriers of the virus with not apparent clinical presentation (3). We are currently conducting a survey among poultry laboratories to evaluate the most important and efficient diagnostic methods utilized to timely and accurate diagnosis of the disease in a flock basis, results from this survey will be presented and discussed.

With the introduction of new viral vector recombinant vaccines (8, 23, 25) and the development of deleted mutants lacking immunogenic glycoproteins (9, 17) raises the possibility to develop serological assays to differentiate vaccinated vs. infected animals (DIVA) will be possible. However the ability of these types of assays to differentiate vaccinated from infected birds needs to be closely evaluated in the field to determine how useful they will be as a flock screening tool.

References

1. Abbas, F., J. R. Andreasen, Jr., and M. W. Jackwood. Development of a polymerase chain reaction and a nonradioactive DNA probe for infectious laryngotracheitis virus. Avian Dis. 40:56-62. 1996.

2. Alexander, H. S., and E. Nagy. Polymerase chain reaction to detect infectious laryngotracheitis virus in conjunctival swabs from experimentally infected chickens. Avian Dis. 41:646-653. 1997.

3. Bagust, T. J. Laryngotracheitis (Gallid-1) herpesvirus infection in the chicken. 4. Latency establishment by wild and vaccine strains of ILT virus. Avian Pathology. 15:581-595. 1986.

4. Callison, S. A., S. M. Riblet, I. Oldoni, S. Sun, G. Zavala, S. Williams, R. S. Resurreccion, E. Spackman, and M. Garcia. Development and validation of a real-time Taqman PCR assay for the detection and quantitation of infectious laryngotracheitis virus in poultry. J Virol Methods. 139:31-38. 2007.

5. Chang, P. C., Y. L. Lee, J. H. Shien, and H. K. Shieh. Rapid differentiation of vaccine strains and field isolates of infectious laryngotracheitis virus by restriction fragment length polymorphism of PCR products. J Virol Methods. 66:179-186. 1997.

6. Creelan, J. L., V. M. Calvert, D. A. Graham, and S. J. McCullough. Rapid detection and characterization from field cases of infectious laryngotracheitis virus by real-time polymerase chain reaction and restriction fragment length polymorphism. Avian Pathol. 35:173-179. 2006.

7. Crespo, R., P. R. Woolcock, R. P. Chin, H. L. Shivaprasad, and M. García. Comparison of Diagnostics Techniques in an Outbreak of Infectious Laryngotracheitis from Meat Type Chickens. Avian Diseases. 51:858-862. 2007.

8. Davison, S., E. N. Gingerich, S. Casavant, and R. J. Eckroade. Evaluation of the efficacy of a live fowlpox-vectored infectious laryngotracheitis/avian encephalomyelitis vaccine against ILT viral challenge. Avian Dis. 50:50-54. 2006.

9. Devlin, J. M., G. F. Browning, C. A. Hartley, and J. R. Gilkerson. Glycoprotein G deficient infectious laryngotracheitis virus is a candidate attenuated vaccine. Vaccine. 25:3561-3566. 2007.

10. Garritty, C. LT: Field Vaccination and dynamics. 42nd National Meeting on Poultry Health and Processing. Oct 8 to 10. Ocean City, Maryland. 2007.

11. Graham, D. A., I. E. McLaren, V. Calvert, D. Torrens, and B. M. Meehan. RFLP analysis of recent Northern Ireland isolates of infectious laryngotracheitis virus: comparison with vaccine virus and field isolates from England, Scotland and the Republic of Ireland. Avian Pathology 29:57-62. 2000.

12. Goodwin, M.A., Smeltzer, M.A., Brown, J., Resurreccion, R.S., Dickson, T.G., Comparison of histopathology to the direct immunofluorescent antibody test for the diagnosis of infectious laryngotracheitis in chickens. Avian Dis 35, 389-91. 1991.

13. Guy, J. S., and T. J. Bagust. Laryngotracheitis. In: Diseases of Poultry 11th ed. Y. M. Saif, H. J. Barnes, J. R. Glisson, A. M. Fadly, L. R. McDougald and D. E. Swayne, eds. Iowa State University Press, Ames, IA. pp 121-134. 2003.

14. Guy, J. S., H. J. Barnes, and L. G. Smith. Rapid diagnosis of infectious laryngotracheitis using a monoclonal antibody- based immunoperoxidase procedure. Avian Pathology 21:77-86. 1992.

15. Han, M. G., and K. S. J. Analysis of Korean strains of infectious laryngotracheitis virus by nucleotide sequences and restriction fragment length polymorphism. Vet Microbiol 83:321-331. 2001.

16. Han, M. G., and S. J. Kim. Efficacy of live virus vaccines against infectious laryngotracheitis assessed by polymerase chain reaction-restriction fragment length polymorphism. Avian Dis. 47:261-271. 2003.

17. Helferich, D., J. Veits, J. P. Teifke, T. C. Mettenleiter, and W. Fuchs. The UL47 gene of avian infectious laryngotracheitis virus is not essential for in vitro replication but is relevant for virulence in chickens. J Gen Virol. 88:732-742. 2007

18. Hughes, C.S., Jones, R.C. Comparison of cultural methods for primary isolation of infectious laryngotracheitis virus from field material. Avian Pathology 17, 295-303. 1988.

19. Humberd, J., M. Garcia, S. M. Riblet, R. S. Resurreccion, and T. P. Brown. Detection of infectious laryngotracheitis virus in formalin-fixed, paraffin-embedded tissues by nested polymerase chain reaction. Avian Dis 46:64-74. 2002.

20. Kirkpatrick, N. C., A. Mahmoudian, D. O'Rourke, and A. H. Noormohammadia. Differentiation of infectious laryngotracheitis virus isolates by restriction fragment length polymorphic analysis of polymerase chain reaction products amplified from multiple genes. Avian Dis. 50:28-34. 2006.

21. Ojkic, D., J. Swinton, M. Vallieres, E. Martin, J. Shapiro, B. Sanei, and B. Binnington. Characterization of field isolates of infectious laryngotracheitis virus from Ontario. Avian Pathology. 35:286-292. 2006.

22. Oldoni, I and M. García. Characterization of Infectious Laryngotracheitis Virus (ILTV) Isolates from United States by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP) of Multiple Genome Regions. Avian Pathology. 36 (2): 167 – 176. 2007.

23. Saif, Y. M., J. K. Rosenberger, S. S. Cloud, M. A. Wild, J. K. McMillen, and R. D. Schwartz Efficacy and safety of a recombinant herpesvirus of turkeys containing genes from infectious laryngotracheitis virus. In: Proc Am Vet Med Assoc. Minneapolis, MN. p 154. 1994.

24. Sellers, H. S., M. Garcia, J. R. Glisson, T. P. Brown, J. S. Sander, and J. S. Guy. Mild infectious laryngotracheitis in broilers in the southeast. Avian Dis. 48:430-436. 2004.

25. Tong, G., S. Zhang, S. Meng, L. Wang, H. Qui, Y. Wang, L. Yu, and M. Wang. Protection of chickens from infectious laryngotracheitis with a recombinant fowlpox virus expressing glycoprotein B of infectious. Avian Pathology 30:143-148. 2001.

26. Van Der Heide, L., Chute, H.L., O'Meara, D.C., 1967. A comparative investigation of the course of an experimental infection with infectious laryngotracheitis in chickens. Avian Dis 11, 149-53.

27. Williams, R. A., M. Bennett, J. M. Bradbury, R. M. Gaskell, R. C. Jones, and F. T. Jordan. Demonstration of sites of latency of infectious laryngotracheitis virus using the polymerase chain reaction. J Gen Virol. 73 (Pt 9):2415-2420. 1992.

28. Williams, R. A., C. E. Savage, and R. C. Jones. A comparison of direct electron microscopy, virus isolation and a DNA amplification method for the detection of avian infectious laryngotracheitis virus in field material. Avian Pathology. 23:709-720. 1994.

Epidemiology of Vaccinal Laryngotracheitis Outbreaks and the Application of Geographic Information Systems (GIS)

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Introduction

Outbreaks of vaccinal laryngotracheitis (VLT) occasionally flare up in broilers, especially in dense production areas. Susceptible broilers are exposed to a virus, usually of chicken embryo vaccine origin, that has a potential to become more virulent and cause disease with bird to bird passages. The virus can then spread between broiler farms within an area. There are many epidemiological factors associated with this disease that provide an understanding of the spread and that can be used as tools for the control of outbreaks. These factors are those associated with the host, the virus and their immediate environment. Looking beyond one affected house, at a group of cases and a pattern of spread, there are additional factors associated with the behaviour of the outbreak, such as those of space, climate and geography. All are closely linked; while the former group of factors have been well defined in the literature, the latter group can be best understood with the use is geographic information systems (GIS), and spatial analysis of outbreak data.

VLT at the farm

Unvaccinated broilers are susceptible to infection starting at 2 weeks of age. Potential sources of the virus are affected, recovered, or chicken embryo origin (CEO) laryngotracheitis vaccinated pullets, breeders and layers, as well as non commercial birds. These birds are potential carriers and may shed the virus to susceptible chickens, especially when under stress. Contaminated litter, equipment and persons can contribute to the spread of the disease from vaccinated, sick, or apparently normal but shedding flocks to susceptible poultry. The virus causing vaccinal laryngotracheitis is a herpes virus that is sensitive to heat and does not survive well outside of the host. The disease has a long incubation period (5-14 days).

Within an affected flock, the disease is highly contagious but spreads slowly and may not express itself in all houses before processing. Birds in the apparently healthy houses may be incubating the disease and shedding virus, contributing to spread. Broiler farms with multiple houses are common and this scenario is easily conceivable on many broiler farms during an outbreak. If infected late, the entire population of a broiler farm may be shedding the virus, without the knowledge of the caretakers. Virus can be detected from the litter of an affected flock, but is destroyed by heat or composting. Within a company, servicemen and other farm visitors, poor biosecurity and contaminated trucks and equipment may contribute to spread.

VLT in an area

Focusing on the epidemiology of an outbreak and the spread of the infection between companies in an area, the factors mentioned above also apply: growers from different companies helping each other on the farm and sharing equipment are an example. Sources of the virus for airborne spread include litter and live haul trucks. Affected, vaccinated or convalescent birds transported to the processing plant may be shedding virus to farms close to the road, contributing to spread. Although the infection is airborne, the virus is heat sensitive. Outbreaks tend to lessen during the

summer. Epidemiological factors can be studied through the use of Geographic Information Systems (GIS) where affected farms and control farms are compared as to their distance to major roads (1), their location within a wind vector of a clinical LT flock (3), and to other farms (1,5). Epidemiological factors can also be ruled out using this technology, such as the proximity of a farm to a water body (1).

Industry control plans and VLT epidemiology

Industry control plans must take into consideration all factors contributing to the spread of VLT into an area, as the first objective is to limit the number of cases. The potential of spread through persons, equipment and fomites are addressed through enhanced communications and biosecurity measures, such as limitations on servicing and visits, and live haul truck cleaning and disinfection. The sensitivity of the virus to heat and outside of its host is addressed through heating the houses, composting the litter and/or 2-3 week downtime periods between flocks, and through a comprehensive litter clean out and litter spreading program (2).

GIS and control strategies

During outbreaks of VLT, GIS is used for applying control strategies, taking these farms and area epidemiological factors into consideration. The computerized maps assist in determining the susceptible population of poultry around a case, the adequate size and shape of biosecurity and vaccination zones, and the routes to the processing plant where the least number of farms are exposed. This powerful tool is also critical in helping understand the epidemiological factors associated to a particular area.

Thoroughly understanding VLT in broiler outbreaks is critical in the control of the disease. The use of GIS assists in effective communications using outbreak maps, helps in making the right decisions for control in a given area.

References

1. Dufour-Zavala, Louise. Use of GIS for Understanding VLT Epidemiology. Presented at AAAP meeting, Honolulu, HI, 2006
2. Dufour-Zavala, Louise. Epizootiology of Infectious Laryngotracheitis and Presentation of an Industry Control Program. Av. Dis 52:1-7. 2008
3. Johnson, Y. J., M. M. Colby, M. S. Myint, S. E. Steele, M. Salem and N. Tablante. Wind-borne transmission of Infectious Laryngotracheitis between commercial Poultry Operations. Int. J. Poult. Sci. 4:263-267. 2005
4. Johnson, Y. J., M. M. Colby, N. L. Tablante, N. Gedamu, B. Gebert, and L. Wilson. Development and Use of geographic information systems (GIS) in the investigation of poultry diseases. In: Proc. 35th National meeting on Poultry Health and Condemnations, Ocean City, MD. Pp. 21-27, 2000.
5. Johnson, Y. J., M. M. Colby, N. L. Tablante, F. N Hegngi, M. Salem, N. Gedamu and C. Pope. Application of commercial and backyard poultry geographic information system databases for the identification of risk factors for clinical infectious laryngotracheitis in a cluster of cases on the Delmarva peninsula. Int. J. Poult. Sci. 3:210-205, 2004.

Infectious Laryngotracheitis Field Perspective: Regional Report, West, How are ILT Breaks Handled?

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Infectious laryngotracheitis (ILT) in broiler chickens and in egg laying chickens was first detected in British Columbia (BC) in 1974 (1) At that time the disease caused significant mortality in susceptible chickens particularly in the Fraser Valley of BC. ILT has been considered endemic in BC since its initial introduction.

Between February and November 2006 and between February and August 2007 there were two outbreaks of ILT in the Fraser Valley of BC. During the 2006 outbreak, 14 farms were positive for ILT and of these, two were repeat farms. During the 2007 outbreak 7 farms were positive for ILT and there were two repeat farms. Three of the positive premises were detected in both the 2006 and the 2007 outbreaks.

The affected farms consisted of pre-vaccinated broiler breeder replacement flocks, broiler flocks, and longer-lived specialty meat chicken flocks. Affected flocks were found to be positive for ILT as early as 4 weeks of age. Mortality rates reach as high as 10 percent on some farms however mortality was often self-limiting or restricted to a single barn or floor. The scheduled ILT vaccination of broiler breeder replacement flocks limited the infection and resulted in a reduction in clinical signs and mortality to normal within 7 days in these flocks.

The gross lesions consisted of mild to severe hemorrhagic laryngotracheitis, with varying amounts of fibrinohemorrhagic exudates. Lungs showed hemorrhagic and patchy congestion and, in some cases, edema. Histopathological examination of affected tissue revealed mucosal necrosis, sloughing, and hemorrhage. Syncitia and basophilic inclusion bodies were also noted. The ILT virus was confirmed by virus isolation and polymerase chain reaction (PCR) tests. PCR and RE-PCR tests were consistent with the presence of chick embryo origin like vaccine. The clinical signs were also consistent with a vaccinal ILT.

Possible risk factors included the proximity to layer flocks where ILT vaccine was administered, and in these cases administered by coarse spray technique. The transport and spreading of manure from layer farms and manure from affected farms near to susceptible farms was considered an important risk factor. Inadequate biosecurity, including inadequate cleaning, disinfection and mortality removal were also suspected as a high risk factor. The possibility of

wind as a factor in carrying and introducing live virus on dust particles was considered a risk factor, especially during the 2007 outbreak.

In Saskatchewan, Alberta and BC, ILT is a provincially reportable disease. Flocks confirmed positive for ILT in Saskatchewan and Alberta are removed. In BC, however, when a positive flock is reported, the industry is notified so that enhanced biosecurity measures can be taken. Affected broiler flocks are transported to processing as soon as possible, taking routes that avoid other farms and ensuring the load is covered as much as possible. All producers of broiler chickens experiencing an ILT outbreak sign a flock attestation form for the processor, so that these broiler chickens will not be subsequently exported to specific markets. In commercial broiler flocks, the use of live vaccine was extremely limited, however, the use of the live vaccine (both embryo derived and tissue culture vaccines) in the longer-lived specialty meat chickens was initiated in subsequent flocks.

In the event of confirmation of a diagnosis of ILT on a farm, in BC, the provincial government will notify the BC Poultry Association (BCPA). The BCPA will in turn notify all members of the BCPA, which include broiler, broiler breeder, commercial layer, commercial layer breeder, turkey, turkey breeder, and specialty poultry associations. The BCPA will provide assistance to the owner of the ILT positive poultry farm, both in terms of providing technical assistance, but also in terms of implementing recommended protocols.

The ILT infected premise will be sent information designed to ensure biocontainment of the disease, or self quarantine procedures. The self quarantine procedures include:

1. Follow the advice of you poultry veterinarian regarding any treatment of your flock.
2. Review and list the on-farm traffic, visitors and bird movements in the previous 24 days. Refer to on farm visitor log.
3. Service unaffected barns first or dedicate a specific employee to the affected barn(s).
4. Immediately restrict on and off-farm access by locking gates and requiring phone-ahead prearrangements for deliveries/pick-ups. Suspend all unnecessary traffic.
5. Follow strict personal biosecurity procedures for leaving the farm (e.g. non-farm clothing, footwear, vehicles, especially if meeting other poultry industry members, even socially.
6. Postpone scheduled vaccinations until discussed with your veterinarian.
7. Postpone or cancel movements of any birds on or off the farm.
8. Dispose of dead/culled birds preferably by on farm incineration or composting. Make sure compost pile is protected from scavenging wildlife. Treat all carcasses as infected material.
9. Call your feed company, egg truck driver, and all other essential services to request "end-of-day" service.
10. Cancel all unnecessary farm visits.
11. Optimal but recommended: Enhanced biosecurity signs posted at gates indicating that an infectious disease has been diagnosed and access is restricted.
12. For broiler producers, continue this self-quarantine until the barn has been thoroughly cleaned and disinfected.
13. For hatching egg and table egg producers, continue this self-quarantine procedure for at least 4 weeks after the last ILT mortality has been observed.

With the biocontainment assured, where an ILT diagnosis has been confirmed, broiler producers are then provided with the following information to minimize the risk of spread.

1. Initiate self-quarantine procedures (as described above).
2. Notify the processing plant of the situation to institute the following action(s):
    a. The affected flock should be the last pick up of the day.
    b. All equipment used for catching should be cleaned and disinfected before leaving the farm.
    c. If weather permits, the trucks should be covered with tarps.
    d. The trucks should use a route to the processing plant that avoids passing other poultry farms, if possible.
    e. Trucks used to haul those birds should be thoroughly cleaned, decontaminated, and disinfected after the birds have been shipped.

3. Make sure the equipment and supplies are available to assist the catchers in cleaning and disinfecting equipment.
4. All mortalities should be incinerated immediately.
5. If mortalities must be composted, make sure that the compost pile is properly covered, and protected from wildlife that may scavenge for carcasses.
6. Manure Management: (recommend no spreading of manure from a positive flock in the Fraser Valley).
    a. After the flock has been shipped, contain the manure in the barn and heat treat to 40° C for 3 days.
    b. Composting needs to be done according to recommendations, preferably heat treatment is done first. Further composting, after heat treatment, will help reduce the risk of virus survival even more. If manure is composted on site, however, it must be well away from the barn and properly prepared and covered.
    c. If manure, after heat treatment, is hauled off the farm, make sure the load is properly secured and covered.
7. Do a completed cleaning and disinfection of the barn:
    a. Wash down the entire inside area, including ceiling, walls, and floor, using a detergent.
    b. Rinse entire inside area down and allow time for drying.
    c. After all surfaces are dry, spray a disinfectant making sure all surfaces are thoroughly coated.
    d. Allow disinfectant to dry before preparing for next flock.

8. Board auditors will be made available to provide direction in the cleaning and disinfection procedures.
9. The BCPA or your veterinarian will follow up with a questionnaire to gather information about this disease outbreak.

With the biocontainment assured, where an ILT diagnosis has been confirmed, hatching egg and table egg producers are then provided with the following information to minimize the risk of spread.

1. Initiate self-quarantine procedures. Maintain self quarantine procedures for at least 4 weeks after the last ILT mortality as been seen.
2. Notify the hatchery or grading station of the situation to schedule your farm as the last egg pick up of the day.
3. ILT vaccination may have to be re-scheduled, therefore, consult with your veterinarian regarding your vaccination program.
4. Have your veterinarian or the Animal Health Centre monitor mortalities at least once weekly until certain no further ILT deaths are occurring.
5. All mortalities should be incinerated immediately.
6. If mortalities must be composted, make sure that the compost pile is properly prepared, covered, and protected from wildlife that may scavenge the carcasses.
7. Do not move live birds off the farm for any reason, including spiking or quota top-up.
8. Manure Management: (recommend no spreading of manure from a positive flock in the Fraser Valley).
    a. After the flock has been shipped, contain the manure in the barn and heat treat to 40° C for 3 days.
    b. Composting needs to be done according to recommendations, preferably heat treatment is done first. Further composting, after heat treatment, will help reduce the risk of virus survival even more. If manure is composted on site, however, it must be well away from the barn and properly prepared and covered.
    c. If manure, after heat treatment, is hauled off the farm, make sure the load is properly secured and covered.
9. Do a completed cleaning and disinfection of the barn:
    a. Wash down the entire inside area, including ceiling, walls, and floor, using a detergent.
    b. Rinse entire inside area down and allow time for drying.
    c. After all surfaces are dry, spray a disinfectant making sure all surfaces are thoroughly coated.
    d. Allow disinfectant to dry before preparing for next flock.
10. Board auditors will be made available to provide direction in the cleaning and disinfection procedures.
11. The BCPA or your veterinarian will follow up with a questionnaire to gather information about this disease outbreak.

The level and thoroughness of producer and allied trade communication and education is considered critical in terms of the successful prevention and control of ILT in BC. To date, March 31 (symposium publication deadline) there have been no new cases of ILT reported in BC.

Infectious Laryngotracheitis International (Latin America) Perspective

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Introduction

Infectious Laryngotracheitis (ILT) has been present in Latin America for several decades. Similarly to the US, there has been an increase in the number of clinical cases as well as in the pathogenicity seen in cases, specially in Mexico.

The purpose of this paper is to present information on the occurrence of ILT in several, but not all , Latin American (LA) countries, on the use of vaccines as well as epidemiological factors involved.

Precise information on the above mentioned, is not easy to obtain since official information is not readily available in most countries, in others, ILT is not reportable. For many, reporting ILT will result in import suspension of poultry products from neighbor and other countries, so private veterinarians are not willing to report clinical cases to the authorities.

The information hereby presented is based mostly on personal communication by veterinarians from different countries who had been personally involved in diagnosis of ILT and or management of infected flocks. Also from consultants to the poultry companies affected. Other information has been obtained from proceedings of poultry conferences in Latin America (LA).

Mexico

ILT appeared in the state of Sonora, northwest of Mexico in the 70s. The origin of the outbreak is believed to have been a flock of layers imported for molting. After that apparently short lived outbreak, the field situation had been free of ILT until the year 2003-04. In that year, mild clinical signs consistent with ILT appeared in a commercial layer flock in the state of Sonora. Lack of experience with the disease and lack of fast communication among poultry veterinarians in the area affected, resulted in live chicken embryo origin (CEO) vaccination of farms belonging to the affected poultry company. It is said that the initial vaccination was done via drinking water. Later, technical poultry meetings resulted in an agreement to circle vaccinate around the outbreaks area and later, all poultry. The break affected mostly commercial layers but also breeders. After the clinical cases subsided, tissue culture origin (TCO) vaccines, via eye drop were used. Actually, CEO and TCO are utilized as a preventive measure. However, some flocks do not include ILT in the vaccination program (4).

Other Poultry areas in Mexico- In 2006, the ILT outbreak spread to other poultry producing states after Sonora. ILT affected the state of Nayarit and Jalisco later, on the same year, the disease spread to most of the poultry producing states such as Puebla, Mexico state, Morelos, Nuevo Leon, Veracruz and even Chiapas (6).

Diagnosis Problems- The outbreak caused a lot of losses in all the poultry producing areas. Lack of experience and knowledge about the disease allowed mistakes to be made. Some of them were

in the diagnosis area and dealing with differentials such as Infectious Coryza (IC), Avian Influenza (AI), Newcastle Disease (ND), Infectious Bronchitis (IB) and others. Field diagnosis without Laboratory confirmation was the most common causes of mistakes.
Mixed viral infections occurred thus, making a clear diagnosis difficult. Sometimes the blame of high mortalities fell on ILT when the real problem was the "other" viral infections. Co-infections of Infectious Laryngotracheitis Virus (ILTV) and Low Pathogenicity Avian Influenza Virus (LPAI) H5N2 also caused serious losses.
Clinical lesions of co-infections of ILTV and MG showed high levels of airsacculitis.
Outbreaks in breeders resulted in severe drops in production as much as 70% drop and 8% mortality. Outbreaks in broilers in the state of Nayarit resulted in 8% or more mortality and 30% or more morbidity (6), (3). Mortality and morbidity rates were much higher when co-infections occurred.
The organization and agreement on vaccination by poultry producers in the state of Nayarit, Mexico, which resulted in control of ILT, even though it took about a year, is an example of what can be achieved by good communication and organization for a common goal.
Reportable Disease- ILT is not a reportable disease in Mexico (6).

Vaccines Used- Initially, live CEO vaccines were used in most of the affected areas. However, the use of these vaccines in the water (low coverage) and the reactions caused by the use of some "very reactive" CEO vaccines, as well as the use in birds older than 2 weeks of age, discouraged some growers since the morbidity and mortality were higher than they are used to see with other viral respiratory vaccines. TCO vaccines were widely used in many poultry areas with apparently good results (3). However, not in others, that had to return to the use of CEO since breaks occurred when TCO cultures were used (area of Tepatitlan, Jal) (2).
Reactions on birds vaccinated with live CEO vaccines that were co-infected with Mycoplasma gallisepticum (MG) caused serious losses. However, ILT breaks on MG positive birds caused still higher losses.
Recombinant Pox –ILT vaccine was used in small amounts for a short period (6).

Typing- The ILT isolates that were sent to the US laboratories for typing showed that most of the isolates were of CEO origin.

Treatments Used- Some flocks with clinical signs of ILT were treated with antibiotics and with mucolytic medication.

Epidemiology- Initially, the spread of ILT from the North West corner of the country to states south of it was due to carrier birds used for molting (7).
However, later on, the spread was due to multiple common factors such as:
1- Proximity of ILTV carrier commercial layers and non-vaccinated broiler farms
2- Live bird markets
3- Transportation of contaminated poultry litter
4- Deficient or lack of biosecurity
5- Deficient drinking water application of live ILT, CEO vaccines
6- Lack of protection of ILT vaccines due to immunosuppression
7- Movement of ILTV carrier replacement commercial pullets to clean areas.
8- Movement of ILTV carrier spent hens to the processing plants or for molting to clean areas
9- Hiding, the presence of the disease in a poultry area

10- Lack of communication among poultry producers and among poultry veterinarians
Actually, ILT in Mexico appears to have become enzootic in many areas of the country

Colombia
ILT was suspected in 1966 in Colombia, however, the first official diagnosis was made in 1970 by "ICA" (officials) in the Dagua county. In 1971, vaccine was approved for use in the affected area. In 1971, an outbreak in Armenia showed mortality to 25% and morbidity to 40%. In 1972, there were outbreaks in the area of El Carmen with mortality up to 69% and 90% morbidity. In 1973, 8 cases were registered in the Cali- Palmira area. In this area the mortalities registered were up to 5.4% and morbidity to 58%. In 1975, two more outbreaks were registered in the Candelaria – Pradera area.
In general all of the cases mentioned above occurred in a area called "Valle del Cauca" which is one of the oldest and more densely populated areas in Colombia (1).
ILT remained enzootic and localized in Valle del Cauca until the late 90's.
In 1998, there was case of mild ILT in commercial layers in the "Sabana of Bogota" area, an area far from the enzootic"Valle del Cauca". At that time authorities and poultry veterinarians decided not to use vaccines since the conclusion at that time was that inappropriate use of live CEO vaccine has played a role in the spread of ILT in "Valle del Cauca", so, they decided to wait and see. The decision worked well without breaks until year 2000. At that time, a different area in the "Sabana de Bogota" suffered breaks so vaccination with CEO and TC vaccines was used widely (7).
The vaccination reduced the case numbers in the area. This second area within "Sabana de Bogota" is made generally of small commercial farmers (5,000 to 10, 000) all in proximity with each other.

TCO outbreaks- In year 2003, ILT outbreaks occurred in the "Santander" area of Colombia. There is an interesting series of outbreaks that deserve comment. After the ILT breaks were more or less under control after using CEO and later TCO vaccines in a different area, some commercial pullet replacements growers utilizing TCO, ILT vaccine in their pullets, were sending the vaccinated birds to "ILT clean areas". The common concept that TCO ILT vaccine viruses will not spread was accepted and so vaccinated pullets were shipped and housed in clean areas. Several breaks occurred, the isolated viruses were typed and 15/16 found to be TCO derived viruses. There was more than one introduction of TCO vaccinated pullets that resulted in outbreaks. These breaks occurred in 2006-2008 (7).

Authorized ILT Vaccines- The use of live CEO, ILT vaccine is illegal in Colombia. The recombinant Pox / ILT and the TCO vaccines are legal.

Observations- Vaccination with CEO, ILT vaccines in the face of outbreaks was attempted many times in Colombia trying to stop the breaks in non previously vaccinated birds. Evidence shown by Delgado (1), indicates that the mortality on flocks vaccinated with CEO in the face of an outbreak had higher mortality than flocks that were affected by ILTV and were not vaccinated in the face of the outbreak.
The combination of ILT, CEO live vaccines and MG infected birds yielded very serious reactions.
Fine spray treatment with disinfectants was used besides antibiotic treatments.

Vaccination with CEO in general showed higher reaction since the pullets were vaccinated when they were over 8 weeks of age or at still older age when the risk of ILT was imminent (1).
Transportation of ILT vaccinated (CEO and TCO) pullets to clean areas has caused outbreaks in Colombia, Transportation of contaminated litter and carrier spent hens to clean areas has also caused breaks (7).
Proximity of farms, lack of communications among farmers regarding disease occurrence and vaccination, vaccine usage in older bird infected or not with MG and other diseases, abundance of back yard flocks in proximity to commercial farms, has favored the spread of ILT (7), (1) .

Costa Rica

There is evidence of ILTV in Costa Rica since 1998. The evidence is from histologically evaluated samples at the School of Veterinary Medicine at the National University of Costa Rica. However, it was not until 2003 that ILT outbreaks were more visible and accepted officially. Histological evidence of breaks between 1996 and 2003 was discarded officially as evidence of occurrence of ILT. Field diagnosis of persistent Infectious Coryza (IC) and treatments with antibiotics as only measure of response, allowed the disease to spread . The breaks in 2003 affected the very dense poultry area of Turrucares de Alajuela. The area contains many broiler and commercial farms in proximity with urban areas with heavy human and vehicle traffic.
Lack of proper diagnosis or acceptance of the histological evidence as well as lack of experience dealing with ILT delayed the proper approach for control (5).
Recognition of the presence of ILT in the country brought up trade barrier from neighboring countries.
In 2005, poultry veterinarians diagnosed several cases of ILT. The affected flocks were mainly commercial layers and were treated with antibiotics, mucolytic medication and by fogging the birds with organic disinfectants. They claim to have seen "satisfactory" results (5).
As a result of a meeting of the National Poultry Association of Costa Rica, live ILT vaccines were banned from use, arguing that live vaccines or their misuse were the cause of the increase in outbreaks. This decision was supported by authorities and the ban remains. Only recombinant Pox- ILT is legal. However, use of this vaccine is limited to commercial layer pullets in the areas of Turrucanes, Piedras Negras and La Guacima. Broiler flocks in the same areas are not allowed to be vaccinated and prevention from breaks is based only on biosecurity. ILT vaccination with the approved recombinant vaccine in broiler breeders, though in small numbers in the mentioned area, is not allowed.
The cost of the Pox-ILT vaccine to the grower is estimated to be $80 US per 1000 doses. The client has to pay 6 weeks in advance to the provider hatchery (5).
A new Poultry Farm Law regulates the sanitary requirements for building and sustaining poultry farms. Together with the decision of not selling baby pullets to small growers by the two main hatcheries in the country, has reduced the numbers of farms that for others were a risk factor for ILT (5).

Comments- ILT has been present in LA for decades. In most cases the breaks occur in clean areas due to import of ILT carrier birds or for illegal use of live virus vaccines in ILT free areas. Transportation of infected manure spreads ILTV. There has been an increase of ILT clinical cases especially in Mexico. Once the disease starts, there is no immediate organized reaction from technically involved people. Immediate vaccination with CEO vaccines without proper planning or common knowledge is widely used. Non-vaccinated poultry neighbors may get "vaccinated" and respond to the clinical signs as it was field case. Directly or "indirectly" ILT

vaccinated birds that are co-infected with Mg or other viral respiratory diseases react severely and the case is considered as field ILT, or the vaccines only is blamed for the reaction.
Live CEO, ILT vaccination in the face of an outbreak resulted in increased mortality in Colombia compared to non-vaccinated.
Evidence of TCO virus causing outbreaks in Colombia deserves further studies.
Field diagnosis without the confirmation of a laboratory may hide or increase the perception of the incidence of ILT or hide the presence of serious illnesses such as AI.
Attempts to hide ILT for export purposes or based on incorrect diagnosis only allows the disease to spread. However, when veterinarians and authorities gather information, study the facts and possibilities, the agreements generally result, independently of type of vaccine used, in reduction and control of cases in that state (Nayarit , Mexico) or country (Colombia and Costa Rica). ILT becomes enzootic in most of the countries where it occurs.

References

1.- García, A. Personal Communication. 2008

2.- Gonzalez C., Laringotraquietis Infecciosa Experiencia en Nayarit Memorias AVECA-G ,Manzanillo ,Colima, 2006

3.-Millan Jorge, Personal Communication, 2008

6.- Mosqueda A. Nuevos criterios en la Prevencion y Control de la Laringotraquietis Infecciosa Aviar. Memorias de APYZAN, Sonora, Mexico, 2006

Lab and Field Perspectives- Diagnostic Tools Available for Diagnosis and Characterization of *Mycoplasma gallisepticum* and Handling of Field Breaks

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DIAGNOSTIC TOOLS

Serology. Serological testing for *Mycoplasma gallisepticum* (MG) infection has not undergone any major recent changes. Flocks are screened primarily by serum plate agglutination, and in the US, reactors are confirmed by hemagglutination-inhibition (HI). Agglutination antigen is available from Intervet America, Millsboro, DE or from Charles River Laboratories, Storrs, CT. Alternatively, screening may be done by ELISA (IDEXX, Westbrook, ME, and Synbiotics, San Diego, CA). Hemagglutination antigen is available through USDA, or commercially from Charles River. Testing of breeding flocks is administered by the National Poultry Improvement Plan (2). Testing procedures for agglutination, and HI are available (2, 21). ELISA testing should be conducted according to the directions of the manufacturer.

The tendency for the serum plate agglutination test to result in false positive reactions is well known (17), and testing should be avoided for the first 4-6 weeks after flocks have been given oil emulsion vaccines. HI testing is highly specific when properly conducted with good quality antigen, but there is antigenic variability among MG strains when tested by HI (22). ELISA test kits are nearly as sensitive as agglutination, but somewhat less specific than HI.

Isolation and identification. Isolation and identification is the "gold standard" for confirming an MG infection. Unfortunately, mycoplasmas grow slowly and require specialized media (21). Isolation may require from 1 up to 4 weeks of incubation. Complications in obtaining field isolates include fastidious strains, low numbers of organisms with low-pathogenic strains and in chronic infections, and overgrowth by mycoplasmal commensals, such as *M. gallinarum* and *M. gallinaceum*.

Polymerase chain reaction (PCR). PCR provides a rapid, sensitive, and specific method for detecting MG DNA. There are many protocols published, but perhaps the most commonly used procedure in the US is that described by Lauerman (25), which is based on the sequence of the 16S rRNA gene. Other recently described procedures are based on the *mgc2* gene (13, 14) or the intergenic spacer region (IGSR) (32). These procedures are roughly equal to the Lauerman procedure in sensitivity and specificity, but sequencing of the PCR product gives useful information for the identification of specific MG strains.

Intraspecies heterogeneity. The ability to distinguish among various MG strains is essential for determining if vaccinated flocks have become colonized with wild-type strains and for strain identification in outbreaks. Until recently we have used a PCR procedure with random primers called random amplified polymorphic DNA (RAPD) (11, 16). The RAPD procedure

scans the entire genome and is quite reliable. Unfortunately, RAPD patterns are not highly reproducible, and RAPD requires a pure culture. More recently we have switched to a diagnostic PCR using primers for the *mgc2* (13, 14) gene and the IGSR (32). The PCR product is then sequenced, allowing the development of a database for comparing sequences of field isolates. Unfortunately, several field strains of MG share the mgc2 sequence with vaccine strain ts-11, while the IGSR sequence is less adept at differentiating among strains with and IGSR sequence of house finch-type isolates. However, the combination of the 2 sequences gives a high probability that different MG strains can be differentiated.

HANDLING MG BREAKS

There are three general aspects of MG control; prevention and eradication, medication, and vaccination.

Control by prevention. Because all of the mycoplasmas pathogenic for poultry, it is essential that replacements be obtained from MG-free sources. A good biosecurity program is essential for maintaining flocks free of infection, especially in areas where there are large concentrations of poultry. Ideally, flocks should be placed in all-in, all-out, single age farms isolated from other poultry flocks and with a good biosecurity program. Ideally, any breeding flock to become infected should be eliminated.

Mycoplasmas are organisms without cell walls, rendering them susceptible to inactivation by environmental factors when outside the host. However, we now know that MG can survive in the environment longer than we previously realized; for example, MG can survive up to 4 days at room temperature on cotton fabric, and on the surface of feathers; it can survive nearly as long on hair, and up to 24 hours in the human nasal cavity (7), showing that movement of humans and their equipment should be carefully controlled. Mycoplasmas are susceptible to a thorough cleanup using any of the common commercially available disinfectants. Experience suggests that the greatest risk factor for horizontal transmission is the presence of infected flocks nearby as is the case with *Mycoplasma hyopneumoniae* in swine herds (29).

An effective, consistently applied monitoring program similar to that used by the NPIP (2) is essential in order to identify infected flocks as early as possible. This is especially important when moving spike males.

Medication. Because mycoplasmas lack a cell wall they are resistant to β lactamic antibiotics such as penicillins or cephalosporins. However, they are susceptible to macrolides, tetracyclines, fluoroquinolones, and others (5, 26, 37). Resistance to antibiotics has also been reported (5, 15, 30, 31). Currently, tylosin or tetracyclines are the most commonly used products in the US for reduction of egg transmission for reduction of egg transmission or prophylactic treatment for the prevention of respiratory disease in broilers or commercial turkeys. Highly effective products such as enrofloxacin or tilmicosin are not approved for use in poultry in the US. Treatment of naturally infected heavy breeders with enrofloxacin was highly effective in reducing upper respiratory tract infection; treated birds were temporarily culture negative for MG (35). Typical medication programs include continuous medication of breeders or layers or perhaps treatment for 1 week each month. In broilers and commercial turkeys, medication is most effective when used prophylactically, typically during the first three days of life and/or at the time of respiratory reactions from viral vaccines.

However, even though medication is an effective tool, it cannot be relied on to completely eliminate infection from a flock (33, 35), and continuous use may result in the development of resistance.

Vaccination. In situations where maintaining flocks free of MG infection is not feasible, usually in muli-age commercial layer operations, vaccination can be a viable option. Choices include inactivated, oil-emulsion bacterins, live vaccines, or a recombinant live poxvirus vaccine containing and expressing key protective MG antigens. Vaccination against MG in poultry has been reviewed by Whithear (38).

Bacterins have been shown to be effective in protecting against respiratory signs and lesions, egg production losses, and in reducing egg transmission (18, 19, 20, 41, 42), but offer little protection against colonization (12). The major advantage of oil-emulsion bacterins is that protection against economic losses can be obtained without the introduction of a live vaccine strain. Disadvantages are cost and the requirement for handling individual birds and relative lack of protection against colonization of field challenge strains of MG.

There are three live vaccine strains licensed in the US, usually for commercial layers; F strain, licensed by Fort Dodge Animal Health (internationally by Schering Plough) and Lohmann Animal Health, 6/85, licensed by Intervet International, and ts-11, licensed in the US by Merial and internationally by Bioproperties, Ltd.

F strain is a naturally occurring field isolate which offers protection against respiratory signs and lesions, egg production losses, and reduces egg transmission (6, 9, 18, 19), but it is too virulent for use in broilers or turkeys (27, 34). It offers protection against colonization (23), and has been used to displace wild-type MG from a multi-age commercial egg operation (36). It is marketed as a lyophilized product and may be administered by spray, eye drop, or in the drinking water.

The 6/85 vaccine strain is a modified US isolate which is apathogenic for chickens and turkeys (10). It was detectable in the upper respiratory tract for 4-8 weeks after vaccination, did not induce a detectable antibody response and was only poorly transmissible (1, 10, 28). The 6/85 vaccine is sold as a lyophilized product, and aerosol administration is recommended.

The ts-11 strain is a chemically mutagenized clone of an Australian field isolate which was selected to grow better at 33C than at 37C. It is safe and efficacious for use in chickens, is apathogenic for turkeys, transmits poorly from bird to bird, and induces a slow antibody response (1, 3, 4, 8, 28, 39, 40). Ts-11 vaccination has been used as a tool for eradication of MG from multi-age commercial layers (36). The ts-11 vaccine is available as a frozen product, and eye drop administration is recommended.

Safety of live MG vaccines is a major concern. For example, F strain has been isolated from clinically ill turkeys (27), and isolates indistinguishable from 6/85 have been isolated from turkeys with clinical MG infection (24). To the author's knowledge, the only isolations of ts-11 vaccine strain from unvaccinated chickens were related to the use of contaminated vaccination equipment.

A recombinant fowl pox vaccine containing and expressing protective MG proteins has been recently introduced by Biomune. There are no published reports concerning its efficacy or safety. However, it has the advantage of not introducing a live MG vaccine strain into a flock. Since no circulating antibodies are detected after vaccination, a serological response would be an excellent indicator of colonization by a wild type field strain.

REFERENCES

1. Abd-El-Motelib, T. Y., and S. H. Kleven A comparative study of Mycoplasma gallisepticum vaccines in young chickens. Avian Dis. 37:981-987. 1993.

2. Anonymous National Poultry Improvement Plan and Auxiliary Provisions. United States Department of Agriculture Animal and Plant Health Inspection Service. APHIS-91-55-063, Washington, DC. 2004.

3. Barbour, E. K., S. K. Hamadeh, and A. Eid Infection and immunity in broiler chicken breeders vaccinated with a temperature-sensitive mutant of Mycoplasma gallisepticum and impact on performance of offspring. Poult. Sci. 79:1730-1735. 2000.

4. Biro, J., J. Povazsan, L. Korosi, R. Glavits, L. Hufnagel, and L. Stipkovits Safety and efficacy of Mycoplasma gallisepticum TS-11 vaccine for the protection of layer pullets against challenge with virulent M. gallisepticum R-strain. Avian Pathol. 34:341-347. 2005.

5. Bradbury, J. M., C. A. Yavari, and C. J. Giles In vitro evaluation of various antimicrobials against Mycoplasma gallisepticum and Mycoplasma synoviae by the micro-broth method, and comparison with a commercially-prepared test system. Avian Pathol. 23:105-115. 1994.

6. Carpenter, T. E., E. T. Mallinson, K. F. Miller, R. F. Gentry, and L. D. Schwartz Vaccination with F-strain Mycoplasma gallisepticum to reduce production losses in layer chickens. Avian Dis. 25:404-409. 1981.

7. Christensen, N. H., C. A. Yavari, A. J. McBain, and J. M. Bradbury Investigations into the survival of Mycoplasma gallisepticum, Mycoplasma synoviae and Mycoplasma iowae on materials found in the poultry house environment. Avian Pathol. 23:127-143. 1994.

8. Collett, S. R., D. K. Thomson, D. York, and S. P. R. Bisschop Floor Pen Study to Evaluate the Serological Response of Broiler Breeders After Vaccination with ts-11 Strain Mycoplasma gallisepticum Vaccine Avian Dis. 49:133-137. 2005.

9. Cummings, T. S., and S. H. Kleven Evaluation of protection against Mycoplasma gallisepticum infection in chickens vaccinated with the F strain of M. gallisepticum. Avian Dis. 30:169-171. 1986.

10. Evans, R. D., and Y. S. Hafez Evaluation of a Mycoplasma gallisepticum strain exhibiting reduced virulence for prevention and control of poultry mycoplasmosis. Avian Dis. 36:197-201. 1992.

11. Fan, H. H., S. H. Kleven, and M. W. Jackwood Application of polymerase chain reaction with arbitrary primers to strain identification of Mycoplasma gallisepticum. Avian Dis. 39:729-735. 1995.

12. Feberwee, A., T. von Banniseht-Wysmuller, J. C. Vernooij, A. L. Gielkens, and J. A. Stegeman The effect of vaccination with a bacterin on the horizontal transmission of Mycoplasma gallisepticum. Avian Pathol. 35:35-37. 2006.

13. Ferguson, N. M., D. Hepp, S. Sun, N. Ikuta, S. Levisohn, S. H. Kleven, and M. García Use of molecular diversity of Mycoplasma gallisepticum by gene-targeted sequencing (GTS) and random amplified polymorphic DNA (RAPD) analysis for epidemiological studies Microbiol. 151:1883-1893. 2005.

14. García, M., N. Ikuta, S. Levisohn, and S. H. Kleven Evaluation and comparison of various PCR methods for detection of Mycoplasma gallisepticum infection in chickens. Avian Dis. 49:125-132. 2005.

15. Gautier-Bouchardon, A. V., A. K. Reinhardt, M. Kobisch, and I. Kempf In vitro development of resistance to enrofloxacin, erythromycin, tylosin, tiamulin and oxytetracycline in

Mycoplasma gallisepticum, Mycoplasma iowae and Mycoplasma synoviae. Vet. Microbiol. 88:47-58. 2002.

16. Geary, S. J., M. H. Forsyth, S. A. Saoud, G. Wang, D. E. Berg, and C. M. Berg Mycoplasma gallisepticum strain differentiation by arbitrary primer PCR (RAPD) fingerprinting. Molec. Cell. Probes 8:311-316. 1994.

17. Glisson, J. R., J. F. Dawe, and S. H. Kleven The effect of oil emulsion vaccines on the occurrence of nonspecific plate agglutination reactions for Mycoplasma gallisepticum and Mycoplasma synoviae. Avian Dis. 28:397-405. 1984.

18. Glisson, J. R., and S. H. Kleven Mycoplasma gallisepticum vaccination: effects on egg transmission and egg production. Avian Dis. 28:406-415. 1984.

19. Glisson, J. R., and S. H. Kleven Mycoplasma gallisepticum vaccination: Further studies on egg transmission and egg production. Avian Dis. 29:408-415. 1985.

20. Hildebrand, D. G., D. E. Page, and J. R. Berg Mycoplasma gallisepticum (MG) — laboratory and field studies evaluating the safety and efficacy of an inactivated MG bacterin. Avian Dis. 27:792-802. 1983.

21. Kleven, S. H. Mycoplasmosis. In: A Laboratory Manual for the Isolation, Identification, and Characterization of Avian Pathogens. L. Dufour-Zavala, D. E. Swayne, J. R. Glisson, J. E. Pearson, W. M. Reed, M. W. Jackwood and P. R. Woolcock, eds. American Association of Avian Pathologists, Athens, GA. pp 59-64. 2008.

22. Kleven, S. H., G. F. Browning, D. M. Bulach, E. Ghiocas, C. J. Morrow, and K. G. Whithear Examination of Mycoplasma gallisepticum strains using restriction endonuclease DNA analysis and DNA-DNA hybridisation. Avian Pathol. 17:559-570. 1988.

23. Kleven, S. H., H.-H. Fan, and K. S. Turner Pen trial studies on the use of live vaccines to displace virulent *Mycoplasma gallisepticum* in chickens. Avian Dis. 42:300-306. 1998.

24. Kleven, S. H., R. M. Fulton, M. Garcia, V. N. Ikuta, V. A. Leiting, T. Liu, D. H. Ley, K. N. Opengart, G. N. Rowland, and E. Wallner-Pendleton Molecular characterization of Mycoplasma gallisepticum isolates from turkeys. Avian Dis. 48:562-569. 2004.

25. Lauerman, L. H. Mycoplasma PCR Assays. In: Nucleic Amplification Assays for Diagnosis of Animal Diseases. L. H. Lauerman, ed. American Association of Veterinary Laboratory Diagnosticians, Auburn, AL. pp 41-52. 1998.

26. Levisohn, S. Antibiotic sensitivity patterns in field isolates of Mycoplasma gallisepticum as a guide to chemotherapy. Isr. J. Med. Sci. 17:661-666. 1981.

27. Ley, D. H., A. P. Avakian, and J. E. Berkhoff Clinical Mycoplasma gallisepticum infection in multiplier breeder and meat turkeys caused by F Strain: Identification by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, restriction endonuclease analysis, and the polymerase chain reaction. Avian Dis. 37:854-862. 1993.

28. Ley, D. H., J. M. McLaren, A. M. Miles, H. J. Barnes, S. H. Miller, and G. Franz Transmissibility of live Mycoplasma gallisepticum vaccine strains ts-11 and 6/85 from vaccinated layer pullets to sentinel poultry. Avian Dis. 41:187-194. 1997.

29. Maes, D., H. Deluyker, M. Verdonck, F. Castryck, C. Miry, B. Vrijens, and A. de Kruif Risk indicators for the seroprevalence of Mycoplasma hyopneumoniae, porcine influenza viruses and Aujeszky's disease virus in slaughter pigs from fattening pig herds. Zentralbl. Veterinarmed [B] 46:341-352. 1999.

30. Migaki, T. T., A. P. Avakian, H. J. Barnes, D. H. Ley, A. C. Tanner, and R. A. Magonigle Efficacy of danofloxacin and tylosin in the control of Mycoplasmosis in chicks

infected with tylosin-susceptible or tylosin-resistant field isolates of Mycoplasma gallisepticum. Avian Dis. 37:508-514. 1993.

31. Pakpinyo, S., and J. Sasipreeyajan Molecular characterization and determination of antimicrobial resistance of Mycoplasma gallisepticum isolated from chickens. Vet. Microbiol. 125:59-65. 2007.

32. Raviv, Z., S. A. Callison, N. Ferguson-Noel, and S. H. Kleven Strain differentiating real-time PCR for Mycoplasma gallisepticum live vaccine evaluation studies. Vet. Microbiol. 129:179-187. 2008.

33. Reinhardt, A. K., A. V. Gautier-Bouchardon, M. Gicquel-Bruneau, M. Kobisch, and I. Kempf Persistence of Mycoplasma gallisepticum in chickens after treatment with enrofloxacin without development of resistance. Vet. Microbiol. 106:129-137. 2005.

34. Rodriguez, R., and S. H. Kleven Pathogenicity of two strains of Mycoplasma gallisepticum in broiler chickens. Avian Dis. 24:800-807. 1980.

35. Stanley, W. A., C. L. Hofacre, G. Speksnijder, S. H. Kleven, and S. E. Aggrey Monitoring Mycoplasma gallisepticum and Mycoplasma synoviae infection in breeder chickens after treatment with enrofloxacin. Avian Dis 45:534-539. 2001.

36. Turner, K. S., and S. H. Kleven Eradication of live F strain *Mycoplasma gallisepticum* vaccine using live ts-11 on a multiage commercial layer farm. Avian Dis. 42:404-407. 1998.

37. Wang, C., M. Ewing, and S. Y. Aarabi In vitro susceptibility of avian mycoplasmas to enrofloxacin, sarafloxacin, tylosin, and oxytetracycline. Avian Dis. 45:456-460. 2001.

38. Whithear, K. G. Control of avian mycoplasmoses by vaccination. Rev. sci. tech. Off. int. Epiz. 15:1527-1553. 1996.

39. Whithear, K. G., Soeripto, K. E. Harrigan, and E. Ghiocas Immunogenicity of a temperature sensitive mutant Mycoplasma gallisepticum vaccine. Aust. Vet. J. 67:168-174. 1990.

40. Whithear, K. G., Soeripto, K. E. Harrigan, and E. Ghiocas Safety of temperature sensitive mutant Mycoplasma gallisepticum vaccine. Aust. Vet. J. 67:159-165. 1990.

41. Yoder, H. W., and S. R. Hopkins Efficacy of experimental inactivated Mycoplasma gallisepticum oil-emulsion bacterin in egg-layer chickens. Avian Dis. 29:322-334. 1985.

42. Yoder, H. W., S. R. Hopkins, and B. W. Mitchell Evaluation of inactivated Mycoplasma gallisepticum oil emulsion bacterins for protection against air sacculitis in broilers. Avian Dis. 28:224-234. 1984.

Experimental Vaccinations for Avian Influenza Virus Including DIVA Approaches

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Introduction

We continue to improve our understanding of avian immunology and are gaining new technological tools that can be used for the immunization of domestic animals. With all these advances we still have to balance the protection that we receive from treatment (i.e vaccination) versus the cost to administer the treatment. The poultry industry is an extremely difficult sector of animal agriculture to vaccinate for because of the low value for individuals birds, the short production lives for most birds, and the costs to produce and administer vaccines. The broiler industry provides the most extreme example with an extremely small margin per bird, extremely short production lives, and the difficulty of vaccinating large numbers of birds once placed in the house. The ideal future vaccines would be available at low cost, be administered by mass administration methods, have no or limited vaccine reactions, allow differentiation of vaccinated from infected animals (DIVA), provide rapid onset of immunity even in the face of maternal or preexisting immunity, provide long-term protection, work in a variety of poultry species when needed, and provide broad cross protection for antigenically variable targets. Current vaccines are far from these ideal goals, and the most optimistic predictions of future vaccines won't live up to all of these expectations. However, currently available and future vaccine technology can provide significant improvements over available vaccines.

So what are the gaps in currently available vaccines and those that are on the near horizon? Of the list of attributes of the ideal vaccine, I will concentrate on two issues that are either a critical need or controversial for the poultry industry, mass vaccination and DIVA vaccination.

Mass Vaccination

Four common methods of mass vaccination for poultry are commonly used including aerosol spray, water, in ovo, and fowlpox vectored vaccines administered by wing web stab at the hatchery. Typically for the spray, water or in ovo routes, a live attenuated vaccine is necessary, which for a virus like Newcastle Disease is readily available with strains like B1 or Lasota. However, no attenuated live vaccines are available for avian influenza because of the concern for reassortment between vaccine and field strains or reversion to virulence. Reassortment is a concern with avian influenza because it is a segmented virus (eight gene segments) that can easily reassort with another influenza viruses if they infect the same cell. This swapping of gene segments can create a virus with new attributes or greater virulence. Reassortment is commonly observed in nature. As with any live vaccines, there is concern about reversion of the virus to virulence. Avian influenza, as a RNA virus has a high mutation rate, would be a likely candidate to revert to virulence. For both these reasons, live vaccines in the past have not been considered favorably as a vaccination choice for avian influenza.

The two types of commercial vaccines available in the U.S. are the killed whole virus oil adjuvanted product and the fowlpox recombinant H5 vaccines. Although both vaccines can provide solid protection from virulent challenge, they both suffer because they can't be administered by a mass vaccination strategy, particularly once birds have been placed in the field. The traditional killed vaccines are administered subcutaneously or by intramuscular injection, which requires each bird to be picked up and vaccinated separately. For birds that are already placed in the field, the cost and manpower required to vaccinate birds, at least for

broilers, makes it almost an insurmountable challenge for routine or even emergency use. Issues about the age that birds can be vaccinated and withdrawal times are also impediments. The fowlpox recombinant H5 vaccines can be administered in the hatchery to day old birds, which provides for more efficient vaccination, but similar issues to the killed vaccines are raised if the birds are already in the field. Additionally, if birds are vaccinated or are naturally exposed to fowlpox, they are resistant to immunization with the influenza part of the vaccine(1).

A third type of vaccine that is available commercially in China and Mexico is a recombinant Newcastle disease virus that also expresses the H5 gene of avian influenza virus. Although the vaccine is licensed in both countries, the level of use and efficacy under field conditions of the vaccine has not been reported. Several different research groups have documented that in specific pathogen free chickens, the NDV vectored H5 vaccine can provide protective antibodies to both NDV and H5 avian influenza(2-6). This type of vaccine is administered as a live vaccine, and can potentially be used by the mass administration routes of aerosol, water, or in ovo vaccination. Two issues remain to be addressed with the NDV vaccine approach. First will pre-existing antibody, either maternal antibody or immune antibody, block the immune response to the vectored vaccine and prevent or reduce the level of antibody to the influenza portion of the vaccine. As previously mentioned, this has been an important issue limiting the value of fowlpox recombinant vaccines to produce a protective immune response to the HA protein(1). The currently described vectored NDV vaccines all appear to only produce low levels of HI antibody to the H5 or H7 insert in SPF birds, and the previous experience with traditional NDV vaccines suggest that pre-existing NDV antibodies may be a serious issue for producing high levels of specific influenza antibody. The second issue relates to the regulatory licensure of the virus in the U.S. As a genetically modified organism (GMO) there will be greater concern about the virus spreading to non-target organisms, and it is likely that any vaccine will be required to be evaluated in a number of different species to be licensed. The primary benefit of this vaccine is that it can be given as a live vaccine that can stimulant humoral, cellular, and mucosal immunity and it transmit bird to bird, but the likelihood of transmission also creates considerable regulatory issues that may make it difficult to license in the U.S.

A new vaccination strategy that is licensed in Mexico has emerged that uses a killed vaccine in a spray vaccination. The killed influenza vaccine is combined with a mucosal adjuvant to generate a mucosal immune response that can effectively provide protection from challenge, at least from a low pathogenic avian influenza (LPAI) challenge. The vaccine, since it is introduced by a spray format, is delivered as small droplets that attach to the lining of the respiratory tract, and the immune response is primarily a mucosal immune response with little serum antibody. For, LPAI the infection is limited to the mucosa, and this type of vaccination should theoretically provide better protection than systemic vaccination (the standard killed adjuvanted subcutaneous vaccines). For highly pathogenic avian influenza, the infection starts by infection at the mucosal surface, but then it spreads systemically. If the immune response to the killed spray vaccine is sufficient, the initial replication can be blocked at the mucosa and the bird will be protected from even HPAI challenge. Currently this type of vaccine provides only low levels of serum antibody, which creates difficulties in easily monitoring the immune response.

Many experimental vaccines are available for potential mass vaccination, including vectored vaccines and live attenuated vaccines. For this discussion, we will divide the vaccines into those replication restricted vaccines and those vaccines that can have autonomous replication. Briefly some of these approaches will be described.

The replication defective vaccines are viruses that are missing a key viral protein that does not allow the viruses to complete the viral life cycle, but the viral particles can still infect like a live vaccine. This allows for both cellular and humoral immunity to be stimulated, but provides the safety of a killed vaccine. No commercial vaccines for poultry are available using this technology, but these types of vaccines have been tested and shown to provide protection in chickens with several different systems. This has included the use of a human adenovirus vector (7, 8) and Venezuelan equine encephalitis (VEE) vector(9). In both systems the viruses are grown in special cell culture lines that allows the complementation of the missing viral proteins. This type of vaccine appears to have great potential in the future, but major obstacles to use remain. The primary obstacle is that because the viruses don't replicate, it requires the administration of a higher level of viral particles to achieve protection. This large amount of viral particles may result in an increased cost per vaccination that is higher than the poultry industry is willing to pay. The second obstacle is the delivery mechanism. Both the adenovirus and alphavirus systems have shown some protection when administered by the in ovo route(7, 9), but neither has demonstrated protection by water or aerosol routes at this time.

The avian influenza attenuated viruses and the replication competent vectored viruses can also be considered together. These viruses have the potential to be produced at low cost and can potentially be administered by mass vaccination means. Because they are live viruses, concerns about reversion to virulence and more specifically with the attenuated avian influenza strains the reassortment of gene segments with other avian influenza viruses remains a major concern. At least three different studies of the development of attenuated avian influenza viruses for use as live vaccine viruses have been reported. All three viruses were produced by reverse genetics with one virus having a cold adapted phenotype similar to the human AnnArbor/68 virus(10), a second having a deletion in the M2 gene(11), and the final virus with change of the HA cleavage sequence from HPAI to LPAI(12). All three viruses protected the experimentally challenged chickens or mice. However, the issue of reassortment of the HA gene with a LPAI and the potential reversion to virulence remains a crucial unresolved issue.

Several other live recombinant vectored viruses and even bacterial vectored vaccines have been experimentally tested. The NDV vectored vaccines have been described previously. Other potential viral vectors include Marek's disease virus, and new generation fowlpox viruses. All the viral vectored vaccines will face similar issues of demonstrating a strong influenza immune response in the presence of antibody to the vector and regulatory issues as a GMO. Several bacterial vectors have also been proposed, including mycoplasma and Salmonella. Concerns about proper glycosylation and protein expression remain as well as regulatory concerns. The live vectored vaccines remain as an area ripe for exploration, but much more work remains to be done.

DIVA Vaccination

The differentiation of vaccinated and infected animals (DIVA) remains a goal for vaccination programs for avian influenza. Currently we have multiple strategies for DIVA vaccination, but none are routinely used. The goal of a DIVA strategy must be clear. One goal would be to clearly separate birds that have been vaccinated from those that are infected. However, from a practical standpoint, that is typically not the information that is needed. The primary reason for DIVA vaccination is to facilitate trade of vaccinated birds. Importing countries want to be assured that poultry or poultry products will not introduce an exotic disease. Currently, serologic testing is extensively used to show that a flock has not previously been exposed to avian influenza because they have no antibody to avian influenza. Even though serology does not give

the current status of the bird, infected or not infected, it has been effective at preventing the introduction of virus between countries. In part because it provides assurance of an active surveillance program in the exporting country, that if transparent to all diagnostic test results , provides a useful assessment of virus presence within a country. Previously vaccinated birds, because they have antibody to most viral gene segments, were barred from most international commerce because the importing country could not by serological means show that the birds were previously free of infection. Although a test like the recently described positive marker test using tetanus toxoid (13) provides greater assurance of the status of the animals as whether infected or vaccinated, it still leaves open the question of whether the flock was vaccinated and then infected. The clear serologic identification of vaccinated and then infected birds is critical if DIVA vaccination for AIV will be a valuable trading tool.

For the killed vaccines three different strategies have been proposed for DIVA testing, the heterologous neuraminidase strategy(14), the nonstructural protein 1 strategy(15), and the M2 strategy(16). Each method has its own potential merits and negatives, and each uses a unique companion diagnostic test to provide the DIVA testing. The subunit vaccines, which expresses only the hemagglutinin and/or neuraminidase genes, can use existing serological tests like AGID or ELISA. The use of sentinel birds also remains an extremely useful surveillance tool in vaccinated flocks.

For all of the DIVA strategies, questions remain about how to apply the methods and interpret the results. None are completely validated, and likely cannot be completely validated because of the differences in viral strains, vaccines, species involved, diagnostic test sensitivity and specificity among some of the bigger variables. Vaccination, by design, will prevent or at least reduce virus shedding. A well matched potent vaccine is likely going to reduce virus shedding to the point where there is little antigen to elicit an immune response, and therefore some or all the birds may not seroconvert. Small amounts of virus shedding, if below the minimum infectious dose, is likely to be of no consequence. However, many vaccines are poorly matched, where large amounts of virus is being shed. This is likely to elicit an antibody response that can easily be detected. However, an intermediate situation may occur where enough virus shedding occurs to have transmission, but not enough to generate a consistent antibody response (17). More research needs to be performed to evaluate these and other potential DIVA strategies to understand when and how they can be applied.

References

1. Swayne, D. E., J. R. Beck, and N. Kinney Failure of a recombinant fowl poxvirus vaccine containing an avian influenza hemagglutinin gene to provide consistent protection against influenza in chickens preimmunized with a fowl pox vaccine. Avian Dis 44:132-137. 2000.
2. Swayne, D. E., D. L. Suarez, S. Schultz-Cherry, T. M. Tumpey, D. J. King, T. Nakaya, P. Palese, and A. Garcia-Sastre Recombinant paramyxovirus type 1-avian influenza-H7 virus as a vaccine for protection of chickens against influenza and Newcastle disease. Avian Dis 47:1047-1050. 2003.
3. Nakaya, T., J. Cros, M. S. Park, Y. Nakaya, H. Zheng, A. Sagrera, E. Villar, A. Garcia-Sastre, and P. Palese Recombinant Newcastle disease virus as a vaccine vector. J Virol 75:11868-11873. 2001.
4. Ge, J., G. Deng, Z. Wen, G. Tian, Y. Wang, J. Shi, X. Wang, Y. Li, S. Hu, Y. Jiang, C. Yang, K. Yu, Z. Bu, and H. Chen Newcastle disease virus-based live attenuated vaccine

completely protects chickens and mice from lethal challenge of homologous and heterologous H5N1 avian influenza viruses. J Virol 81:150-158. 2007.
5. Steel, J., S. V. Burmakina, C. Thomas, E. Spackman, A. Garcia-Sastre, D. E. Swayne, and P. Palese A combination in-ovo vaccine for avian influenza virus and Newcastle disease virus. Vaccine 26:522-531. 2008.
6. Veits, J., D. Wiesner, W. Fuchs, B. Hoffmann, H. Granzow, E. Starick, E. Mundt, H. Schirrmeier, T. Mebatsion, T. C. Mettenleiter, and A. Romer-Oberdorfer Newcastle disease virus expressing H5 hemagglutinin gene protects chickens against Newcastle disease and avian influenza. Proc Natl Acad Sci U S A 103:8197-8202. 2006.
7. Toro, H., D. C. Tang, D. L. Suarez, M. J. Sylte, J. Pfeiffer, and K. R. Van Kampen Protective avian influenza in ovo vaccination with non-replicating human adenovirus vector. Vaccine 25:2886-2891. 2007.
8. Gao, W., A. C. Soloff, X. Lu, A. Montecalvo, D. C. Nguyen, Y. Matsuoka, P. D. Robbins, D. E. Swayne, R. O. Donis, J. M. Katz, S. M. Barratt-Boyes, and A. Gambotto Protection of mice and poultry from lethal H5N1 avian influenza virus through adenovirus-based immunization. J Virol 80:1959-1964. 2006.
9. Schultz-Cherry, S., J. K. Dybing, N. L. Davis, C. Williamson, D. L. Suarez, R. Johnston, and M. L. Perdue Influenza virus (A/HK/156/97) hemagglutinin expressed by an alphavirus replicon system protects chickens against lethal infection with Hong Kong-origin H5N1 viruses. Virology 278:55-59. 2000.
10. Song, H., G. R. Nieto, and D. R. Perez A new generation of modified live-attenuated avian influenza viruses using a two-strategy combination as potential vaccine candidates. J Virol 81:9238-9248. 2007.
11. Watanabe, T., S. Watanabe, J. H. Kim, M. Hatta, and Y. Kawaoka Novel approach to the development of effective H5N1 influenza A virus vaccines: use of M2 cytoplasmic tail mutants. J Virol 82:2486-2492. 2008.
12. Shi, H., X. F. Liu, X. Zhang, S. Chen, L. Sun, and J. Lu Generation of an attenuated H5N1 avian influenza virus vaccine with all eight genes from avian viruses. Vaccine 25:7379-7384. 2007.
13. James, C. M., Y. Y. Foong, J. P. Mansfield, S. G. Fenwick, and T. M. Ellis Use of tetanus toxoid as a differentiating infected from vaccinated animals (DIVA) strategy for sero-surveillance of avian influenza virus vaccination in poultry. Vaccine 25:5892-5901. 2007.
14. Capua, I., C. Terregino, G. Cattoli, F. Mutinelli, and J. F. Rodriguez Development of a DIVA (Differentiating Infected from Vaccinated Animals) strategy using a vaccine containing a heterologous neuraminidase for the control of avian influenza. Avian Pathol 32:47-55. 2003.
15. Tumpey, T. M., R. Alvarez, D. E. Swayne, and D. L. Suarez Diagnostic approach for differentiating infected from vaccinated poultry on the basis of antibodies to NS1, the nonstructural protein of influenza A virus. J Clin Microbiol 43:676-683. 2005.
16. Lambrecht, B., M. Steensels, S. Van Borm, G. Meulemans, and T. van den Berg Development of an M2e-specific enzyme-linked immunosorbent assay for differentiating infected from vaccinated animals. Avian Dis 51:221-226. 2007.
17. van der Goot, J. A., M. van Boven, G. Koch, and M. C. de Jong Variable effect of vaccination against highly pathogenic avian influenza (H7N7) virus on disease and transmission in pheasants and teals. Vaccine 25:8318-8325. 2007.

The Role of Vaccines and Biosecurity in Control of H3N2 Swine Influenza Infection in Turkey Breeder Flocks

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Type A influenza virus infection in turkeys results in clinical signs ranging from asymptomatic to severe. Symptoms may include respiratory disease, drop in egg production, reduced hatchability, eggshell abnormalities, decreased feed efficiency, and increased mortality. In 2003, a H3N2 subtype of influenza triple reassortant containing human, swine, and avian gene segments, was isolated from turkey breeders in North Carolina housed in close proximity to a swine operation. This flock experienced a severe drop in egg production, egg quality, and hatchability, with no other clinical signs. Subsequently, additional turkey breeder flocks from areas of concentrated swine production in North Carolina, Ohio, Minnesota, Indiana, Missouri, and Canada were infected with the H3N2 subtype of influenza. Commercial turkey flocks also tested serologically positive for H3N2, but showed no clinical signs and performed normally. This presentation will discuss the association of index cases with large swine units, diagnostic procedures, biosecurity measures taken, and vaccination strategies to control introduction of the virus.

A vaccine trial conducted at a BSL3Ag facility (USDA-ARS-Southeast Poultry Research Laboratory, Athens, GA) compared egg production, egg quality, and virus isolation results from turkey breeder hens vaccinated with either a monovalent H3N4 commercial oil emulsion inactivated vaccine, a bivalent H3N2 / H1N1 oil emulsion inactivated autogenous vaccine, or sham-vaccinated, followed by challenge with a 2003 turkey field isolate of H3N2 influenza. Following a natural route of challenge (eye drop / intranasal), hens vaccinated with the H3N2 / H1N1 autogenous vaccine were significantly protected from drops in egg production observed in hens vaccinated with commercial H3N4 or sham-vaccinated. Hens receiving either the commercial or autogenous H3 subtype vaccines had decreased numbers of both unsettable eggs and virus isolations from cloacal swabs, compared to the sham-vaccinated hens.

Turkey and chicken ovaries were subjected to lectin-binding studies to determine the presence of receptors utilized by influenza viruses. Influenza isolates containing mammalian-origin hemmaglutinin proteins preferentially bind sialic acid α2,6-galactose (SAα2,6gal) linked receptors, while avian-origin hemmaglutinin proteins bind sialic acid α2,3-galactose (SAα2,3gal). Turkey ovaries exhibited strong reactions for both SAα2,3gal and SAα2,6gal

linked receptors, while chicken ovaries only showed strong reactions for SAα2,3gal. Because these H3N2 turkey isolates contain a mammalian-origin hemmaglutinin, the presence of the SAα2,6gal linked receptors in turkey ovaries provides the molecular platform for attachment and infection. This finding also implicates turkeys as a potential mixing vessel for generating influenza reassortants of mammalian and avian origin in the field. It may also help to explain the absence of reports of H3N2 subtype influenza infection in broiler breeders and table egg layers in areas of concentrated swine production.

References

Campitelli L., C. Fabiani, S. Puzelli, A. Fioretti, E. Foni, A. De Marco, S. Krauss, R.G. Webster and I Donatelli. H3N2 influenza viruses from domestic chickens in Italy: an increasing role for chickens in the ecology of influenza? Journal of General Virology 83:413-420. 2002.

Choi, Y.K., J.H. Lee, G. Erickson, S.M. Goyal, H.S. Joo, R.G. Webster, and R.J. Webby. H3N2 influenza virus transmission from swine to turkeys, United States. Emerging Infectious Diseases 10:2156-2160. 2004.

Ficken, M.D., J.S. Guy, and E. Gonder. An outbreak of influenza (H1N1) in turkey breeder hens. Avian Diseases 33:370-374. 1989

Gonder, E. A novel avian influenza outbreak in H1N1-vaccinated turkey breeders. International Turkey Reproduction Symposium October 1-3, 2003, Raleigh, NC.

Hinshaw, V.S., R.G. Webster, W.J. Bean, J. Downie, and D.A. Senne. Swine influenza-like viruses in turkeys: potential source of virus for humans? Science, 220:206-208. 1983.

Olsen, C.W. et. al. Triple reassortant H3N2 influenza A viruses, Canada, 2005. Emerging Infectious Diseases. 2006 Jul 1.

Olsen, C.W., S. Carey, L. Hinshaw, and A.I. Karasin. Virologic and serologic surveillance for human, swine, and avian influenza virus infections among pigs in the north-central United States. Arch. Virol. 145:1399-1419. 2000.

Suarez, D. L., P.R. Woolcock, A.J. Bermudez, and D.A. Senne. Isolation from turkey breeder hens of a reassortant H1N2 influenza virus with swine, human, and avian lineage genes. Avian Diseases 46:111-121. 2002.

Tang, Y., C.W. Lee, Y. Zhang, D.A. Senne, R. Dearth, B. Byrum, D.R. Perez, D.L. Suarez, and Y.M. Saif. Isolation and characterization of H3N2 influenza A virus from turkeys. Avian Diseases 49:207-213. 2005.

Tilley, B., E. Gonder, C. Smith, and S. Jackson. Swine flu (H3N2) in turkey breeders. AVMA July 15-19, 2006, Honolulu, HI.

Wright, S.M., Y. Kawaoka, G.B. Sharp, D.A. Senne, and R.G. Webster. Interspecies transmission and reassortment of influenza A viruses in pigs and turkeys in the United States. American Journal of Epidemiology 136:488-497.

Yassine, H.M., M.Q. Al-Natour, C.W. Lee, and Y.M. Saif. Interspecies and intraspecies transmission of triple reassortant H3N2 influenza A viruses. Virology Journal 4:129, 2007.

Yassine, H.M., C.W. Lee, D. L. Suarez, Y. M. Saif. Genetic and antigentic relatedness of H3 subtype influenza A viruses isolated from avian and mammalian species. Vaccine. 2007, Dec 26.