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An Episode of

Infectious Laryngotracheitis

Affecting Mississippi Broiler-Breeders and Broilers in 2002-2003

(Includes 1995 ERAD Manual)

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Abbreviations: CAS = chorioallantoic sac, CAM = chorioallantoic membrane, CEO = chicken-embryo origin (vaccine), FA = fluorescent antibody, HA = hemagglutinating/hemagglutination, ILT = infectious laryngotracheitis, IBV = infectious bronchitis virus (Ark = Arkansas, Conn = Connecticut, Mass = Massachusetts strains of IBV), GIS = geographic informational system, GPS = global positioning system, MBAH = Mississippi Board of Animal Health, MLV = modified-live virus, NDV = Newcastle disease virus, PCR = polymerase chain reaction, PBS = phosphate-buffered saline, SPF = specific-pathogen-free, TCO = tissue culture origin (vaccine), TPB = tryptose phosphate broth, VI = virus isolation

An Episode of Infectious Laryngotracheitis Affecting Mississippi Broiler-Breeders and Broilers in 2002-2003

SUMMARY

After nearly 20 years of freedom from clinical infectious laryngotracheitis (ILT), the Mississippi poultry industry experienced a limited, somewhat confined, episode of the disease that lasted a little more than 3 months (December 11, 2002, to March 17, 2003). The index case was detected in broiler-breeder chickens and subsequently was seen in broiler flocks. During the episode, 65 suspect cases that fit our case definition were submitted to the laboratory. Of these cases, 32 were diagnosed as ILT positive by a combination of embryo passage, histopathology, and fluorescent antibody techniques.

The episode was controlled and ultimately eradicated by the collaboration of three components—the commercial poultry companies and their growers, the state regulatory authority for animal diseases, and the veterinary diagnostic laboratory services of the state—actively working together. Overall efforts focused on increased surveillance, increased attention to biosecurity guidelines, and the use of modified-live virus (MLV) vaccines. Once it was apparent that more than one premises was affected, chicken-embryo origin (CEO) vaccines were permitted and required in a defined zone that surrounded the first few ILT-positive cases. However, within a short period, a number of cases occurred outside that zone (and several considerably distant from the zone). Accordingly, the decision was made to abandon the zone approach in favor of requiring CEO vaccination of all broilers within the state with a few exceptions. One geographically isolated company that grew only broilers to less than 6 weeks of age and the USDA-ARS and Poultry Science Department farms located at Mississippi State University were exempt.

During the course of the episode, existing biosecurity guidelines were modified as necessary, and others were implemented as indicated by circumstances. Of particular concern was the potential that residual virus would remain on ILT-positive premises and those premises on which the CEO vaccine had been used. Accordingly, a protocol was developed for the treatment of these houses and the movement of the litter originating from these premises.

The last ILT-positive case was submitted to the laboratory on March 17, 2003. A month and half later, on May 9, all use of CEO ILT vaccines was suspended in the state. From that time until this writing (December 2006), no diagnosis of ILT was made within the state.

BACKGROUND

Prior to December 2002, the last known case of clinical infectious laryngotracheitis (ILT) in Mississippi occurred during the winter of 1981-82. Although specifics of that episode were not documented, Thornton and Sadler were active then and remember it as being somewhat similar in certain respects to the one reported here. That is, the disease lasted only a couple of months; it was believed to have been caused by a vaccine-related virus; it involved more broiler than breeder or layer flocks; and it was controlled by the industry's agreeing to vaccinate their breeders with TCO ILT vaccine and their broilers with CEO ILT vaccine. There were two differences between the two cases: (1) the 1981-82 episode was confined to a smaller area—primarily around Interstate 20 in the area of Morton to Forest, Mississippi; and (2) the use of CEO vaccines in broilers was limited to only those flocks within a 1-mile radius of an ILT-positive farm and never involved statewide vaccination of broilers.

The initial case in the 1981-82 episode occurred in a flock of ILT-vaccinated leghorn pullets that were brought in from neighboring Alabama. Some believe that the disease was precipitated by the stress of the movement and the extremely cold temperatures that were present during that movement. In hopes of containing the problem, that flock was sold as soon as the diagnosis was confirmed. However, shortly thereafter, two breeder flocks broke with ILT. Both had connections with the stressed ILT-vaccinated leghorns that broke. One of the breeder flocks had laborers that also worked on the leghorn farm and the other received a gas delivery from a truck that had been on the ILT-positive leghorn farm. Signs in the breeder birds were severe and included the presence of blood clots on the wall of the chicken house and considerable mortality. The leghorn flock, the two breeder flocks, plus some seven to eight broiler flocks, which were ILT positive, constituted the extent of the 1981-82 episode. The policy at that time—to require TCO ILT vaccination of all breeder flocks in the state and CEO ILT vaccination of all commercial poultry within a 1-mile radius of any ILT-positive farm—is credited with containing that episode in a matter of weeks.

There was a limited recurrence of ILT in the state 1 year later. The very next flock of birds on one of the two breeder farms involved in the 1981-82 episode broke with the disease, even though it had been vac-

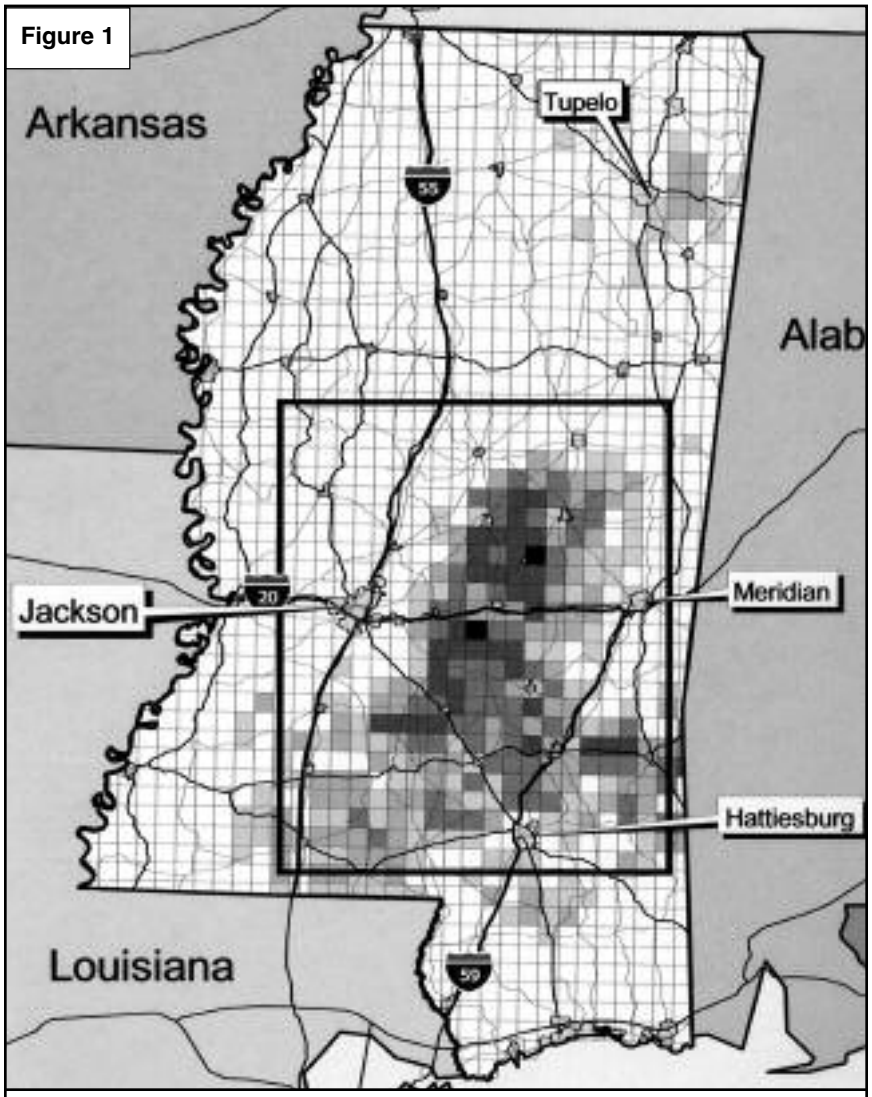


Figure 1

Map of Mississippi showing the grid system used to plot the location of broiler farms within the state in 1999 (scale: 1:2,800,000). Shades of gray denote those grids with commercial broiler farms; darker shades indicate the higher capacities of chickens. (The large, overlaid rectangle outlines the area shown in more detail in Figures 2 A–C.)

cinated with TCO ILT. Also, a replacement flock of leghorn pullets—also vaccinated with TCO ILT—was split between four layer farms, each of which subsequently broke with the disease. The growers on these five farms were advised to take their birds out of production, to increase their level of biosecurity and biocontainment, and to maintain the birds until they were deemed safe to transport. After a reasonable period, the birds from all five farms were sent to slaughter. No further spread of the disease was detected.

One of the residual memories of the 1981-82 episode was that, although modified-live (MLV) ILT vaccines were effective in controlling the disease, CEO vaccines, in particular, had the potential to exacerbate the problem. With this in mind, the poultry industry of Mississippi voluntarily agreed that the use of all MLV ILT vaccines should be avoided. Accordingly, the use of all CEO vaccines was prohibited at the conclusion of the 1981-82 episode and then again at the end of the 1982-83 episode. The use of TCO vaccine in breeders continued for about another 4 to 5 more years before it was prohibited as well. (A primary breeder company maintained a facility within the state during that time and, because of the high level of biosecurity it exercised, was permitted to use TCO vaccine until it ceased operations in December 1999.)

In spring 1999, conditions changed. At that time, numerous cases of ILT were being diagnosed in surrounding states, and CEO ILT vaccine was being used in adjacent Alabama. Because of the relation of the northern segment of the Mississippi poultry industry to affected poultry in Alabama (Figure 1), and because of that segment's isolation from the main Mississippi poultry industry located in the central and southern part of the state, CEO vaccine was permitted for broiler operations in the northern segment. Furthermore, because of the threat presented by the use of CEO vaccines both within the state and in other nearby states, it was deemed advisable to re-permit the use of TCO vaccines for Mississippi's breeder population. After only a few months, the threat of ILT in Alabama had subsided, so in July 1999 no further use of CEO was permitted in Mississippi. However, the use of TCO in the state's breeders remained and was still in effect as of January 2006.

This bulletin documents the specifics of the ILT episode that occurred in Mississippi during the winter months of 2002-03. It also includes the measures that were used to control it and observations of what can be done for future episodes.

Materials and Methods

Bird and Tissue Samples

Most cases were received as morbid or freshly dead birds. Tissues were collected at necropsy and submitted for virus isolation and histopathological examination. For the first few cases, only tracheas were collected; however, shortly into the episode, eyelids and lungs were also collected. Kidneys and cecal tonsils were collected from many cases and submitted as well. The number of specimens varied, but in most cases the clinicians submitted tissues from a minimum of five birds per case. Tissues for histopathological examination were placed in 10% buffered-neutral formalin. Tissues for virus detection that could be transported to the virus laboratory on the day collected were held at 5°C; otherwise, they were frozen at -20°C and transported as soon as possible.

On a few occasions, company personnel collected and submitted tissues directly for virus detection and histopathological examination.

Histological Procedures

A single transverse section was trimmed from each trachea, and a single full-length (dorsal to ventral) section was trimmed from each eyelid perpendicular to the lid margin. Multiple transverse sections were trimmed from each lung, and the section that contained the largest bronchus was selected. All tissues were then processed routinely by serial dehydration in ethanol and infiltrated with xylene and paraffin, embedded in paraffin blocks, sectioned at 5-6 µm, and stained with hematoxylin and eosin.

As part of virus isolation procedures, pieces of embryonic chorioallantoic membranes (CAM) collected from embryos inoculated with field material were submitted for histological examination. These tissues were placed into cassettes, as submitted, without further trimming or manipulation and processed, embedded, sectioned, and stained as outlined previously.

Tissue Preparation for Microbiological Analysis

Trachea (together with any lung and lower eyelid tissues submitted) were combined and are considered in this report as "respiratory tissues." Other tissues, primarily kidneys and cecal tonsils, were combined separately and considered here as "nonrespiratory tissues."

Tracheas were opened longitudinally and any excess exudate and debris was removed. The tracheal mucosa was then scraped and the contents placed in 5 ml of tryptose phosphate broth (TPB). If submitted, lungs and lower eyelids were rubbed vigorously with cotton swabs and that material added to the tube containing the tracheal scrapings. After vortexing, tubes containing respiratory tissues were sampled for *Pasteurella multocida*, *Ornithobacterium rhinotracheale*, *Bordetella avium*, *Mycoplasma gallisepticum* (MG), and *M. synoviae* (MS) and then frozen (-65°C). Of the nonrespiratory tissues, kidneys were stabbed with a cotton swab; ceca were opened and cleaned of excess cecal contents, and the mucosa in the area of the cecal tonsils was swabbed. Kidney and cecal swabs were combined in a separate 5 ml tube of TPB and frozen (-65°C).

After three freeze/thaw cycles, the contents of tubes containing sample tissues were passed through an 0.22-µm syringe filter, diluted 1:10 in TPB containing 100 IU/ml of penicillin G and 100 mg/ml of streptomycin, and allowed to stand at 22°C for 45 minutes before embryo inoculation.

Detection of Specific Aerobic Bacteria and Mycoplasma in the Respiratory Tract

A loop-full of original sample tube containing respiratory tissues was inoculated onto a 5% sheep blood agar (BA) plate (BBL, Becton Dickinson and Company, Cockeysville, Maryland). The plate was incubated at 37°C in a CO_2 incubator. After 2 days the plate was inspected. Small colorless colonies containing Gram-negative pleomorphic rods were analyzed for cytochrome C oxidase and catalase activity, motility, growth on MacConkey agar, indole production, ability to reduce nitrate, and their reactions on an API 20NE® test strip (bioMérieux Vitek, Inc., Hazelwood, Missouri). Identification was based on comparing these results with those published in the literature (10,22).

The tube of combined respiratory tissues was also analyzed for the presence of *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS). An aliquot of the original sample TPB tube was passed through a 0.45-µm syringe filter and the filtrate inoculated into MG and MS broth (6). Inoculated tubes were incubated at 37°C for 21 days. During that period, the tubes were compared daily with uninoculated control tubes of the same media for any color change in the phenol red indicator. Any tube turning color was subcultured onto MS agar (6). These plates were incubated for a minimum of 7 days at 37°C in a candle jar. At the end of that period, the plates were viewed using a dissecting microscope. Any plate showing mammiform colonies typical of *Mycoplasma* morphology were considered positive and the origin of the positive broth tube, MG or MS, used to determine the species.

Virus Isolation and Identification

The tube of respiratory tissues was used to detect the presence of infectious laryngotracheitis (ILT) virus. Seven 11-day-old embryonated eggs from a commercial specific-pathogen-free (SPF) source (Hy-Vac, Inc., Gowrie, Iowa) were inoculated via the chorioallantoic membrane (CAM) route using 0.2 ml of the antibiotic-treated sample/egg. The eggs were sealed, incubated at 37°C, and candled daily. Those eggs containing live embryos 6 days later were opened and their CAMs examined. CAMs containing plaques or similarly suspicious lesions were harvested and pieces placed into McDowell's fixative (17) for histological examination. Pieces of affected CAMs also were placed into phosphate-buffered saline (PBS) and submitted for fluorescent antibody (FA) evaluation for ILT virus (Dr. Frank Austin, College of Veterinary Medicine, Mississippi State University). The remaining portions of the affected membranes were held frozen (-60°C). If either the histological or FA reports were positive for ILT virus, no additional testing was conducted; otherwise, the membranes were thawed, ground, subjected to three freeze-thaw cycles (frozen to -65°C), and then passed onto the CAMs of additional eggs for a second pass. To be considered negative, four or more embryos had to survive the 6-day postinoculation period and have no visible lesions on their CAMs.

Additional embryonated eggs were inoculated to detect the presence of other viral pathogens, including infectious bronchitis virus (IBV), Newcastle disease virus (NDV), and avian influenza virus (AIV). For this, equal amounts of both respiratory and any nonrespiratory tissues submitted were combined and served as the inoculum. Five 11-day-old SPF embryonated eggs were inoculated via the chorioallantoic sac (CAS) route

using the same dose of inoculation and same conditions of incubation as stated above. After 2 days of incubation, two eggs were removed from incubation. Their CAMs were harvested for IBV FA (Dr. Frank Austin, College of Veterinary Medicine, Mississippi State University) and their CAS fluids harvested for polymerase chain reaction (PCR) amplification for the same virus. Four days later—after 6 days of incubation—an aliquot of CAS fluid was sampled from all remaining embryos and pooled. The embryos were then removed and examined for any lesions. The CAS fluid of any embryo dying during the 6-day incubation period was sampled separately. An aliquot of all sampled CAS fluids was inoculated into tubes of TPB and incubated at 37°C to detect bacterial contamination. In addition, an aliquot of sampled CAS fluids was mixed with an equal volume of 10% chicken red blood cells in PBS to determine the presence of hemagglutination (HA) agents. Hemagglutination-negative eggs were opened and their embryos examined for lesions. Harvested CAS fluids were frozen (65°C) and thawed three times and then inoculated for any further CAS passages. CAS fluid from embryos that died in the previous passage and that were free of bacterial contamination were added to the pool of CAS fluids and used as the inoculum for the next passage. To be considered negative, CAS-passaged samples were passed a total of four passages without evidence of any lesions or HA activity.

If IBV was identified in the first passage CAS fluid, one additional CAS passage was made to make sure that no HA-positive agent was also present. In the absence of any embryonic mortality or morbidity with suggestive lesions, positive IBV FA or PCR, or HA activity, each submission was passed in embryos for a total of four 6-day passages before being considered negative. Occasionally, a case that had been IBV-negative by PCR on pass 1 produced IBV-like lesions—dwarfing, stunted down, reflexively bent toes—in higher passages. When this occurred, one additional CAS passage was made and, after 2 days of incubation, CAS material was harvested and tested by PCR for IBV.

The agar gel precipitin (AGP) test was used to confirm the identity of other viruses. Harvested CAS fluid with HA activity was tested against virus-specific antiserum to NDV (SPAFAS, Inc., Norwich, Connecticut) and AIV (USDA, Ames, Iowa). CAS fluids harvested from embryos with lesions suggestive of IBV were also tested against adenovirus Group I antiserum (SPAFAS, Inc.). Once loaded, AGP plates were placed in a humidified container, incubated at 22°C, and inspected after 24 and 48 hours of incubation for precipitin lines. Known positive antigens were included on all plates and served as positive controls.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Identification of IBV Isolates

Ribonucleic acid (RNA) extraction was performed as previously described (8) with some modifications. Briefly, 250 µl of CAS fluid was centrifuged at 1,310 X *g* for 15 minutes. Two hundred microliters of the supernatant was mixed with 15 µg of glycogen and 1 ml of TRIzol® reagent (GIBCO BRL, Grand Island, New York) and vortexed for 15 seconds. The solution was mixed with 220 µl of chloroform, vortexed for 15 seconds, and centrifuged at 11,750 X *g* for 5 minutes. The RNA in the aqueous phase was precipitated with 750 µl of isopropanol for 30 minutes and centrifuged at 11,750 X *g* for 10 minutes. The pellet was washed with 75% ethanol, air dried, and then dissolved in 6 µl of diethyl pyrocarbonate-treated distilled water. The GeneAmp RNA PCR kit (Perkin Elmer Cetus, Norwalk, Connecticut) was used for reverse transcriptase (RT) PCR. The PCR for cDNA was amplified using serotype-specific primers described by Keeler, et al. (15). The PCR products were separated by electrophoresis in 1.5% agarose containing 0.01% GelStar (FMC BioProducts, Rockland, Maine).

Location and Size of Poultry Industry

In 1999, following a respiratory outbreak involving IBV (18) that had occurred the year before, the poultry companies in Mississippi were given a map of the state overlaid with a 6 X 6-mile grid system and asked to report the number of farms and the capacity of those farms located in each grid. Once received, their responses were collated and entered into an electronic database. Summary results from that database showed that there were 8 companies (most of which had more than 1 grow-out complex) and some 2,000 farms actually involved in the raising of commercial poultry in the state (18).

Conversations with several individuals associated with the state's poultry industry revealed that the relative increase in poultry housing over the intervening years (1999 to 2003), was considered to be slight. Therefore, the distribution of the poultry industry during the ILT episode is considered to be reasonably similar to the 1999 map (Figure 1).

Regulatory Activities

In Mississippi, the MBAH is the state agency responsible for animal health issues. Within that agency, the state veterinarian and three poultry epidemiologists carry out the policies that affect poultry production. In addition to supervising the depopulation of the index case, the MBAH was particularly active in the regulatory efforts to control the episode. Once ILT was reported in the state, their initial efforts were devoted to alerting the various components of the poultry industry in the state to be aware that the disease was present and to increasing surveillance for the disease. This continued throughout the episode, as the various aspects of the state's poultry industry were kept abreast of the episode as it developed. MBAH also recirculated existing biosecurity guidelines that had been drafted previously by the poultry industry, and after consultation with other health professionals, implemented other guidelines as seemed necessary and advisable. Attention was also paid to external groups that had direct contact with either the poultry farms or company facilities and personnel to make them aware of the ILT's presence and to provide them training in biosecurity and the need to adhere to established biosecurity measures. This involved individuals associated with industries allied to poultry as well as staff of federal and other state agencies whose duties required them to be on farm premises.

In Mississippi, no live ILT vaccine may be used unless permitted by the state veterinarian. As soon as it was established that ILT had spread into broilers, the state veterinarian established a zone around the ILT-affected farms and required all broilers within that zone to be vaccinated with CEO ILT. The zone was possible because of a previous collaboration between the state veterinarian, the College of Veterinary Medicine, and the Center for Advanced Spatial Technology in Agriculture (ASTA) of Mississippi State University in which global positioning system (GPS) longitudes and latitudes of most poultry farms in the state were collected and entered into a geographical information system (GIS) software program (ARCVIEW, ESRI, Inc., Redlands, California). Later it became necessary to require statewide vaccination of all broilers with CEO ILT. (Note: Due to its remote location relative to other commercial poultry in the state and due to some of its farms being within Alabama, one company located in a southeastern portion of the state was exempt from this requirement.) All activities related to using these vaccines and coordinating the starting and the stopping of vaccination were coordinated by MBAH.

As the episode progressed, there was concern about the residual level of ILT virus on ILT-positive farms and farms on which CEO ILT vaccine had been used. In response, MBAH established guidelines for the treatment of litter on these farms. Specifically, once a farm was free of birds, the growers and their company supervisors were requested to (1) immediately be sure that all live and dead birds had been removed; (2) immediately wind-row the litter, then close the house and heat its interior to 100°F for 3 days; (3) adhere to the MBAH litter movement permit process (more in another section of this bulletin); (4) clean and disinfect the interior of the house; and (5) try to provide 2 weeks of downtime before the next flock is delivered. MBAH did permit variances, allowing some companies to shorten downtimes or by-pass heating. These variances resulted in no ill effects being noted. It was determined that the best method of handling broiler breeder (hen) farms diagnosed as ILT positive or vaccinated with CEO ILT vaccine was not to remove the birds within 2 weeks following a diagnosis of ILT or CEO vaccination as the potential for transmission and spread of the virus would be inordinately higher during that period.

Related to the aforementioned house/litter guidelines, MBAH also established a litter permitting system to restrict the removal of litter from these farms. The system required that interested parties request permission from the state veterinarian before removing the litter. The MBAH's primary role in this process was to ensure, as much as possible, that the route being used to transport the litter had minimal contact with other poultry farms.

Biosecurity Practices Questionnaire

Approximately 1 year after the episode, each of the companies growing commercial poultry in the state was sent a set of questionnaires to determine the biosecurity practices they had exercised before, during, and after the ILT episode. At the time of the episode, 8 different companies were growing commercial poultry in the state. Several companies had more than 1 grow-out division. In all, there were a total of 14 such divisions in the state at that time. For purposes of the questionnaires, the individuals contacted were those responsible for health decisions within their division and/or company. (Some companies were organized so that 1 individual was responsible for these decisions for all of its

divisions, while others were organized such that each of the company's divisions acted independently of each other.) Accordingly, a total of 10 sets of questionnaires were sent to individuals representing their company/divisions.

The sets of questionnaires were divided into three parts (Appendices A-C). The first two parts were similar; one asked about biosecurity practices in the company's pullet/breeder operations, and the other asked about biosecurity practices in their broiler operations. Most questions in these two parts were structured to be answered "yes" or "no" with a request to provide a written explanation for any "yes" response. Also, many of the questions were asked in such a manner that the respondents could respond to whether the particular practice/activity was a change from what had been done before the episode and whether that practice/activity was being maintained after the episode had passed. The third part of the questionnaire set asked two questions designed to discover the industry's overall impression of how the episode was handled: (1) *In your opinion, what was done correctly to diagnose, control, and eradicate the problem?* and (2) *In your opinion, what could have been done to improve the diagnosis, control, and eradication of the problem*? Those respondents whose responses were either illegible or unclear were contacted for clarification.

INDEX CASE

On December 11, 2002, 8 live and 12 dead 63-week-old broiler-breeder birds from a commercial poultry operation were submitted to the diagnostic laboratory. The primary complaint was that the birds were experiencing labored breathing, the birds were cyanotic, and the flock had increased mortality. At the time of the problem, the farm contained approximately 21,300 hens and 1,900 roosters housed in two houses. Of these, the problem was confined to the male birds in only one of the two houses on the farm. The most consistent lesions seen on necropsy were caseous tracheitis, caseous airsacculitis involving the heart and lungs, and consolidation of the lungs. No hemorrhage was noted in the tracheas of these birds. Although the main complaint was about the male birds on this farm, egg production was also affected. According to company records, the hens came into production during the spring of 2002 and peaked at a level of 81%. Just prior to the onset of respiratory signs, production was at a level of 52% but then fell to 31% as the disease progressed.

Assorted tissues, including trachea and lung, were collected for histological examination. Microscopic inspection of these tissues revealed subacute necrotizing pneumonia and subacute proliferative tracheitis, with intranuclear inclusions visualized in both the lung and trachea. Based on the history, signs, and gross and microscopic lesions, a presumptive diagnosis of infectious laryngotracheitis (ILT) was made. This was later confirmed by CAM inoculation and FA testing of tissues from this same farm. Bacteriological analysis detected *Pasteurella multocida* and *Escherichia coli* from the airsac lesions and lungs of these birds.

Following the advice of representatives of other poultry companies, the affected company decided to destroy the 17,900 hens and 1,570 roosters remaining on the affected premises. This was done by CO_2 overdose on December 27 and 28. The birds were herded into about a third of their respective houses, partitioned off with plastic sheeting, and killed using liquid CO_2. Afterwards, the carcasses were buried nearby on land leased from another individual. A 20-foot-deep trench was dug, and the birds were placed in it, covered with lime, and buried. MBAH personnel were in attendance during these procedures.

Total cost of depopulation and burial, less labor, was estimated to be $27,300 (including $20,000 lost from not processing the birds, $1,800 cost of CO_2, $5,000 leasing of land, and $500 rental of equipment). Indemnity was neither sought nor was any paid for this action; the contracting company bore all expenses.

Results of All Cases in the Episode

Case Definition and Cases Received

For purposes of this bulletin, a case is defined as any laboratory submission received from commercial broiler or pullet/breeder flocks between December 11, 2002, and March 17, 2003, in which birds were experiencing respiratory problems—ocular discharge, nasal discharge, snicking, gasping, open-mouth breathing, or respiratory rales—and had not been vaccinated with ILT CEO vaccine. The definition is further refined to eliminate any repeat submissions from the same flock of chickens. Within this case definition, ILT-positive cases are those that were confirmed positive by the laboratory methods described herein.

Including the index case, 65 cases were received during the 96-day period (December 11, 2002, through March 17, 2003) that fit this definition. These cases originated from all 8 commercial broiler production companies (designated herein as Companies A through H) growing poultry in the state at that time (Table 1).

Of the 65 cases, 13 were submitted from breeder flocks and 52 from broilers. One of the companies (Company A) accounted for 8 of the 13 breeder flock submissions and the only 3 ILT-positive breeder flock cases. The remaining 5 breeder flock cases, all of which were ILT negative, originated from Companies C, E, and H. Of the 52 cases that originated from broiler flocks, 32 were ILT positive. Three companies (A, B, and C) accounted for the largest number of these cases and the highest number of ILT-positive cases. Company A submitted 16 broiler cases, of which 9 were ILT positive; Company B with 17 broiler submissions had 8 ILT-positive cases; and Company C with 10 broiler submissions also had 8 ILT-positive cases. Three other companies (D, E, and F) accounted for 6 broiler submissions and the remaining 4 ILT-positive broiler cases, while the 2 remaining companies (G and H) submitted 3 broiler cases, of which none were ILT positive.

Table 1. Cases submitted from commercial poultry companies in Mississippi during an episode of infectious laryngotracheitis (ILT) that occurred between December 11, 2002, and March 17, 2003.

Company	Breeder cases		Broiler cases	
	Submitted	ILT positive	Submitted	ILT positive
A	8	3	16	9
B	–	–	17	8
C	1	0	10	8
D	–	–	2	1
E	1	0	2	2
F	–	–	2	1
G	–	–	2	0
H	3	0	1	0

Signs and Lesions in ILT-Positive Cases

The impression is that as the episode started in breeder birds, it was fairly mild, but then it spread fairly rapidly into broilers where the lesion severity increased. While the index case was typical of what was reported for the other breeder cases, broilers experienced more severe lesions that progressed as the problem spread. Those in the field reported a marked dyspnea, which could be heard as a distinct whistling sound from outside the chicken house. Inside the house, it was more customary for all birds in the house to be affected, rather than those in one particular section. Many of the affected birds had watery eyes, had swollen sinuses/heads, and were clinically depressed. "Blood slinging," or the presence of blood clots on the walls of the chicken houses, was not reported by either growers or company service personnel until later in the episode and then only rarely. Mortality would generally be higher than normal in the first affected house of a multi-house farm, but as the problem spread to other houses on the farm, it tended to get worse with the final house(s) being the most severely affected in terms of both morbidity and mortality. Mortality figures as high as 8% per house were noted on some affected farms.

At necropsy, almost all broilers had varying degrees of conjunctivitis, periocular swelling, and tracheitis, all suggestive of typical respiratory disease. The conjunctivitis occasionally was accompanied with keratitis and corneal ulceration, indistinguishable from that due to high levels of ammonia (19). Tracheal lesions could vary widely within the same flock, ranging from erythema and edema to a catarrhal or to a necrotic exudate that sometimes occluded the lumen. Tracheal hemorrhage, as typical of virulent ILT (3), was not a prominent finding, and with the exception of some breeder chickens, pneumonia was mild or absent. Infrequently, birds would present with typical airsacculitis lesions (pericarditis and perihepatitis).

Confirmation of ILT

Of the 65 total cases received, 55 were examined by histology and virus isolation (VI) (that also involved histological and/or FA examination, herein referred to as "VI-plus"), 7 were examined by histology alone and 3 by VI-plus alone. Of the 32 ILT-positive cases, 1 case was examined by VI-plus procedures only, and 31 cases were examined by both direct histology and by VI-plus procedures. Of these, there was complete agreement between both direct histology and by VI-plus procedures for 24 cases, 6 cases were histologically positive and VI-plus negative, and 1 case was histologically negative and VI-plus positive.

On an experimental basis, another technique, cytology, was evaluated for its effectiveness in diagnosing ILT. (See sidebar, *Cytology: A Faster Technique for Diagnosing ILT?*)

Table 2. Additional microbiological agents isolated from 65 cases of commercial broilers and (broiler) breeders submitted during the December 11, 2002, to March 17, 2003, episode of ILT in Mississippi.

Additional agents	ILT positive	ILT negative
Other viruses		
Arkansas-infectious bronchitis virus (IBV)	7	7
Connecticut IBV	8	1
Massachusetts IBV	3	[1][1]
Newcastle disease virus (NDV)	[2][2]	1
Adenovirus	[1][3]	1
none	14	23
Total	**(32 cases)[4]**	**(33 cases)[4]**
Bacteria		
Bordetella avium	7	7
Pasteurella multocida[5]	2	0
Ornithobacterium rhinotracheale	1	2
overgrown (*Proteus spp.*)	2	2
not examined	3	4
none	17	18
Total	**(32 cases)**	**(33 cases)**

[1]Detected in 1 of the Arkansas IBV cases.
[2]Detected in 1 of the Arkansas IBV cases and in 1 of the Connecticut cases.
[3]Detected in 1 of the Connecticut IBV cases.
[4]Does not include numbers in brackets.
[5]Only additional microbe detected in breeders.

Histopathological Findings

Histologically, there was erosion and ulceration of the tracheal mucosa in association with epithelial syncytia, epithelial intranuclear inclusion bodies, and mild to moderate lymphohistiocytic and heterophilic inflammatory infiltrates. Comparatively few cells contained distinct intranuclear inclusion bodies, but many of the syncytial cells had pale basophilic glassy nuclei with thin rims of marginated chromatin, which is suggestive of inclusions. Syncytial cells with intranuclear inclusion bodies were also seen in the luminal exudate. In addition, the tracheal submucosa was irregularly thickened with edema and mild mixed inflammatory infiltrates.

A few of the ILT-positive cases produced generalized, diffuse thickenings on the CAMs of inoculated eggs, while most produced one or several individual plaques measuring about 1 cm in diameter. Both the diffuse thickenings and the individual plaques had a yellowish tinge, the same hue as the yolk. Histologically, these CAMS were mostly characterized by moderate to marked stromal hyperplasia, stromal heterophilic inflammation, and epithelial hyperplasia with epithelial necrosis, syncytia, and intranuclear inclusion bodies.

Other Microbiological Agents

Other viruses were detected in the broiler submissions (Table 2). A total of 27 IBV were detected, of which 14 were determined to be Arkansas type, 9 were Connecticut type, and 4 were Massachusetts type. Newcastle disease virus was isolated from 3 cases and adenovirus was isolated from another 2 cases. The distribution of these non-ILT viruses was generally similar between the 32 ILT-positive and the 33 ILT-negative cases. However, it should be noted that 8 Connecticut IBV were detected in the ILT-positive cases as opposed to 1 in the ILT-negative cases.

The only significant bacterium isolated from the 13 breeder bird cases was *Pasteurella multocida*. The organism was detected twice, and both times it occurred in the ILT-positive birds of Company A—once from the index case birds received on December 11, 2002, and once from a case submitted during the period from January 9 to January 31, 2003. Bacteria of suspected association with respiratory diseases were isolated from several of the broiler cases. *Bordetella avium* was isolated from 14 cases, and *Ornithobacterium rhinotracheale* was isolated from 3 cases. The numbers of these isolates were fairly evenly distributed between the ILT-positive and ILT-

negative cases. No *Mycoplasma* was isolated from any of the cases submitted.

Nine of the 65 cases had combinations of the viruses and bacteria mentioned above associated with them. This included the three IBV strains identified with either *B. avium* or *O. rhinotracheale* and adenovirus with *O. rhinotracheale*. The main distribution difference was that 4 cases of Arkansas IBV + *B. avium* were detected in the ILT-negative cases, while only 1 case of Arkansas IBV + *B. avium* was detected in the ILT-positive cases.

Table 3. Chronological distribution of cases of infectious laryngotracheitis (ILT) diagnosed in Mississippi broilers and (broiler) breeders between December 11, 2002, and March 17, 2003.

Time period[1]	Breeder cases		Broiler cases	
	Submitted	ILT positive	Submitted	ILT positive
12/11/02	1	1	0	0
12/12/02 – 1/8/03	3	0	9	4
1/9/03 – 1/31/03	4	2	21	9
2/1/03 – 3/17/03	5	0	22	16
Total	**13**	**3**	**52**	**29**

[1]From 4/10/99 to date, tissue culture origin (TCO) ILT vaccine was permitted for breeders in Mississippi. (Chicken embryo origin [CEO] vaccine was briefly permitted for broilers grown in the northern part of the state during April and June of 1999.) From 1/9/03 through 1/31/03, a zone was established around the ILT-positive premises and CEO ILT vaccine was required in broilers and permitted in breeders. From 2/1/03 through 5/09/03, unrestricted use of CEO ILT vaccine was required statewide; afterwards, it was prohibited.

Chronology of the Episode and the Use of ILT Vaccines

For the purposes of this bulletin, this episode is divided into four time periods: December 11, 2002; December 12, 2002, to January 8, 2003; January 9, 2003, to January 31, 2003; and February 1, 2003, to March 17, 2003 (Tables 3 and 4 and Figure 2 A-C).

December 11, 2002. This date contained the submission of the index case, which was detailed earlier in this bulletin.

December 12, 2002 – January 8, 2003. Twelve additional suspect cases (3 from breeders and 9 from broilers) had been submitted, of which no breeder flock cases and 4 broiler flock cases were diagnosed as ILT positive. Following conversations with the poultry companies, the state veterinarian established a triangular-shaped zone, which encompassed 4 of the 5 ILT-positive premises to date. (The location of the fifth case was considered reasonably isolated from other poultry premises and was handled as an individual vaccine site.) The zone contained some 200 square miles, primarily within Leake and Neshoba counties. The outline of the zone was as follows: starting at Carthage, Mississippi, north on State Highway 35; then north on State Highway 25 to the community of Four Corners; then south on State Highway 19 to Philadelphia, Mississippi; then west on County Road 488 to the community of Laurel Hill; then west on Laurel Hill Road and then Galilee Road until it rejoins County Road 488; then west on County Road 488 to its junction with State Highway 35; then north on State Highway 35, back to Carthage (Figure 2A). The companies growing broilers in this zone were required to vaccinate all flocks with CEO ILT vaccine, and this was done when the birds reached approximately 14 days of age. Also, companies growing breeders in the zone were permitted to boost their TCO-vaccinated birds with the CEO vaccine if they strongly desired to do so. However, because of the severity of the CEO ILT vaccine and its propensity toward persistence, this practice was highly discouraged. The vaccine zone became effective on January 9, 2003.

January 9, 2003 – January 31, 2003. During this period, the number of cases submitted and the number of ILT-positive cases increased. A total of 25 cases were received, 4 from breeder and 21 from broiler flocks. Of these, 2 of the breeder cases and 9 of the broiler cases were ILT positive. As January progressed, several ILT-positive cases were occurring outside of the vaccine zone (Figure 2B), and plans were being made to enlarge that zone on its southern border to encompass them. However, after the 14th and 15th ILT-positive broiler cases occurred well south of I-20, more than 45 miles from any other ILT-positive premises to date, the decision was made to abandon the vaccine zone approach and require statewide CEO vaccination of all broilers. This occurred on February 1. At that point, CEO vaccine was again permitted as a boost for TCO-vaccinated breeders statewide; however, this practice was strongly discouraged.

February 1, 2003 – March 17, 2003. During this period, 27 cases were received during this period. Five of these were from breeders, none of which were ILT positive, and the other 22 cases were from broilers, of which 16 were ILT positive. The last ILT-positive case fitting the case definition was received on March 17, 2003.

Initially, CEO ILT vaccines were given at "full dose" (i.e., one dose per bird) via the drinking water route following manufacturer's recommendations. Due to the need to vaccinate farms as quickly as possible with available manpower, the vaccines were usually applied by company service personnel. Shortly into the episode, several companies noted heavier-than-acceptable respiratory reactions after water vaccination. They subsequently evaluated and then changed to spray application. By trial and error, most discovered that somewhere between a half and one full dose of vaccine per bird reduced the degree of respiratory reaction to an acceptable level.

On the basis of no additional ILT-positive cases after March 17 and the general lack of respiratory problems noted in the field, the decision was made to stop permitting the use of CEO vaccine within the state on May 9, 2003. From that point on, the state resumed the pre-episode policy, which involved only vaccination of breeder replacements with TCO ILT vaccine.

Looking at the composite of all ILT-positive cases (Figure 2C), it can be seen that the episode was confined to the southern portion of the state's poultry industry and did not involve the poultry grown in the smaller area located in the northern part of the state. Within the southern portion, the

Table 4. Individual infectious laryngotracheitis (ILT)-positive cases occurring in Mississippi broilers and (broiler) breeders during the December 11, 2002, to March 17, 2003, episode.

Time period[1]	Case	Day collected[2]	Company code[3]	Type bird	Age (days)	Same grid as case[4]
12/11/02	1	12/11/02 (0)	A	breeder	441	–
12/12/02 – 1/8/03	2	1/6/03 (26)	A	broiler	49	20
	3	1/6/03 (26)	B2	broiler	29	12
	4	1/7/03 (27)	A	broiler	33	–
	5	1/7/03 (27)	A	broiler	39	11
1/9/03 – 1/31/03	6	1/10/03 (30)	A	broiler	46	–
	7	1/14/03 (34)	A	breeder	266	–
	8	1/14/03 (34)	A	broiler	39	–
	9	1/21/03 (41)	A	breeder	392	–
	10	1/21/03 (41)	B1	broiler	56	–
	11	1/22/03 (42)	A	broiler	36	5
	12	1/27/03 (47)	A	broiler	45	3
	13	1/27/03 (47)	B1	broiler	55	17, 30
	14	1/28/03 (48)	A	broiler	42	–
	15	1/29/03 (49)	C1	broiler	35	21
	16	1/30/03 (50)	D	broiler	52	19, 23
2/1/03 – 3/17/03	17	2/3/03 (54)	B1	broiler	38	13, 30
	18	2/5/03 (56)	E1	broiler	40	–
	19	2/6/03 (57)	B1	broiler	45	16, 23
	20	2/6/03 (57)	B1	broiler	46	2
	21	2/7/03 (58)	C1	broiler	50	15
	22	2/7/03 (58)	C2	broiler	56	–
	23	2/10/03 (61)	A	broiler	32	16, 19
	24	2/14/03 (65)	B1	broiler	21	–
	25	2/17/03 (68)	E2	broiler	38	–
	26	2/24/03 (75)	F	broiler	38	–
	27	2/24/03 (75)	C3	broiler	61	–
	28	2/24/03 (75)	C3	broiler	57	–
	29	2/27/03 (78)	C3	broiler	46	–
	30	2/28/03 (79)	B2	broiler	51	13, 17
	31	3/12/03 (91)	C3	broiler	61	–
	32	3/17/03 (96)	C1	broiler	61	–

[1]Correspond to time periods in Table 3.
[2]Number in parenthesis is days after index case collected (12/11/02).
[3]Numbers indicate different divisions within same company.
[4]Grids refer to accompanying map (Figure 2).

ILT-positive cases could be further divided into two clusters—one located north of Interstate 20 and the other located south of I-20, with some 30 miles separating the two clusters. Chronologically, the first 13 ILT-positive cases appeared in the northern (north of I-20) cluster, primarily in the Carthage-Philadelphia-Forest area. Cases #14 and #15 occurred south of I-20, well south of those in the northern cluster. The remaining 16 cases were located in one or the other of these two clusters. By the end of the episode, the northern cluster contained 23 ILT-positive cases, and the southern cluster contained 9 ILT-positive cases.

Figure 2C also shows that many of the ILT-positive cases in the northern cluster tended to be close together and located in grids that contained higher concentrations of poultry, while in the southern cluster, the ILT-positive cases were more spread apart and did not occur in the more concentrated poultry areas. It is interesting to note that the concentrated grid located just southwest of Forest, Mississippi—and approximately halfway between both ILT clusters—had no ILT-positive cases.

Additional (Noncase Definition) Cases

Twenty-five additional cases were received during the time period of this episode, and although they do not fit the case definition, they are included here for informational purposes. As soon as ILT was diagnosed on the index farm, the company involved collected samples from 4 more of its breeder farms. One of these submissions was diagnosed as ILT positive; however, there was never any morbidity or mortality in these birds to suggest clinical ILT disease. Also, 14 cases of broilers from CEO-vaccinated flocks were submitted during the episode. Six of these cases were submitted during the February 1, 2003, to March 17, 2003, time period, and all were ILT positive. Eight of these cases were submitted after the episode (between March 18, 2003, and May 15, 2005), none of which were ILT positive.

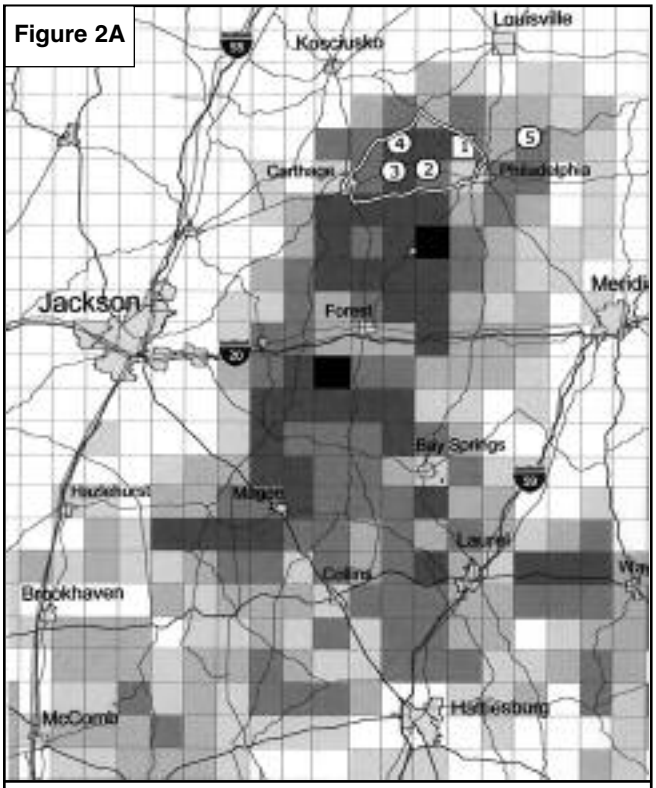
Six cases (1 from a breeder flock and 5 from broiler flocks) were considered suspicious of ILT by company personnel and dealt with internally without submitting them for laboratory confirmation. Communications with these companies disclosed that the breeder flock had been vaccinated with TCO vaccine, while none of the 5 broiler flocks had received any ILT vaccination. Dates given for when these flocks appeared ill were January 9 (3 of the broiler flocks), February 14 (2 broiler flocks), and February 20 (the 1 breeder flock).

During the episode, we also had occasion to receive and examine 1 case of commercial laying pullets and 3 cases of "backyard-type" chickens, none of which were found to be ILT positive.

Responses to Questionnaires about Biosecurity Practices

Of the 10 sets of questionnaires sent out, 8 companies responded (Appendices A-C). Of these, all but 2 of the 14 company/divisions (complexes) in the major (southern) portion of the state's poultry industry were represented. Based on the approximate size of each of the companies, it is estimated that the respondents represented approximately 90% of that area's poultry industry.

Figure 2A



Closer view of the southern portion of the Mississippi poultry industry showing the distribution of ILT-positive cases (scale: 1:1,200,000). Numerical markers are used to indicate the progression of the ILT-positive cases. Of these, ILT-positive breeder cases are outlined in squares, and positive broiler cases are outlined in ovals. Note that the location of the premises is indicated by the grid within which it is located and does not indicate the exact location. The time periods mentioned are those listed in Table 4. Figure 2A shows the index and first four ILT-positive cases that occurred during the first two time periods (December 11, 2002, to January 8, 2003). The vaccine zone established on January 9, 2003, is outlined in white. (The fifth case was vaccinated and handled as an individual premise outside of the vaccine zone.)

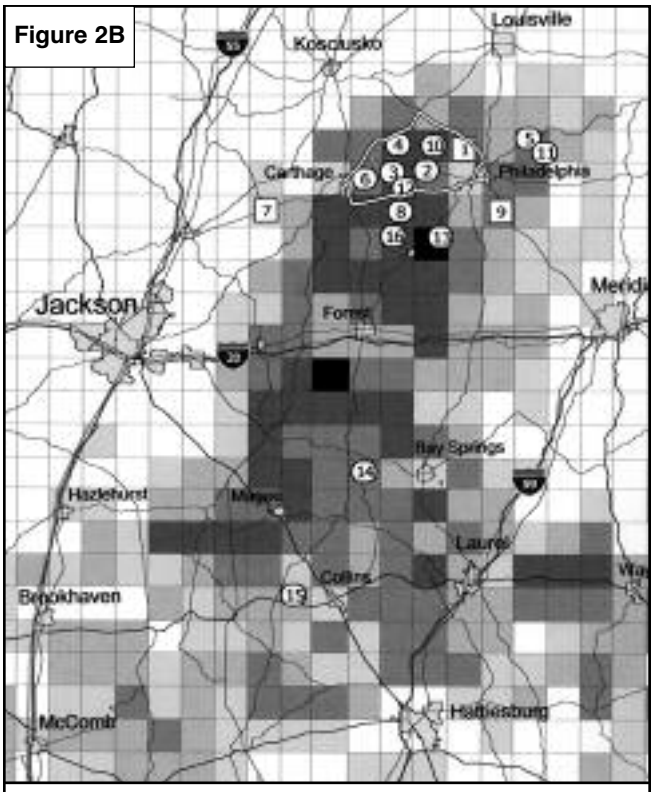
Specific responses to the questionnaires can be found in the Appendices. Appendix A contains the responses about pullet/breeder biosecurity practices; Appendix B contains the responses about broiler biosecurity practices; and Appendix C lists the respondents' opinions about what had been done correctly and what could have been done better to diagnose, control, and eradicate ILT.

Based on these responses and comments from industry personnel, it appears that prior to this episode, there were generally two levels of biosecurity in place: a higher level for pullet/breeder farms and a lower level for broiler farms. Once it was realized that ILT was in the state, the industry increased the level of biosecurity on both types of farms. On pullet/breeder farms this included making sure that company biosecurity policies were being carried out by the growers, discouraging growers from being in physical contact with each other, placing more restrictions on their own service personnel to minimize the transmission potential of them and their vehicles, and severely limiting the access of non-company (e.g., repair, utility, construction, and installation) personnel to the farms. In addition, those companies that used CEO vaccine in their pullets attempted to minimize contact between vaccinated pullets and any nonvaccinated pullets and their mature breeder flocks.

The level of biosecurity on broiler farms substantially increased during the episode to the extent that it more approximated the level in place on the pullet/breeder farms during this period. Those companies that used CEO ILT vaccine also made an effort to prevent contact between their vaccinated and nonvaccinated farms.

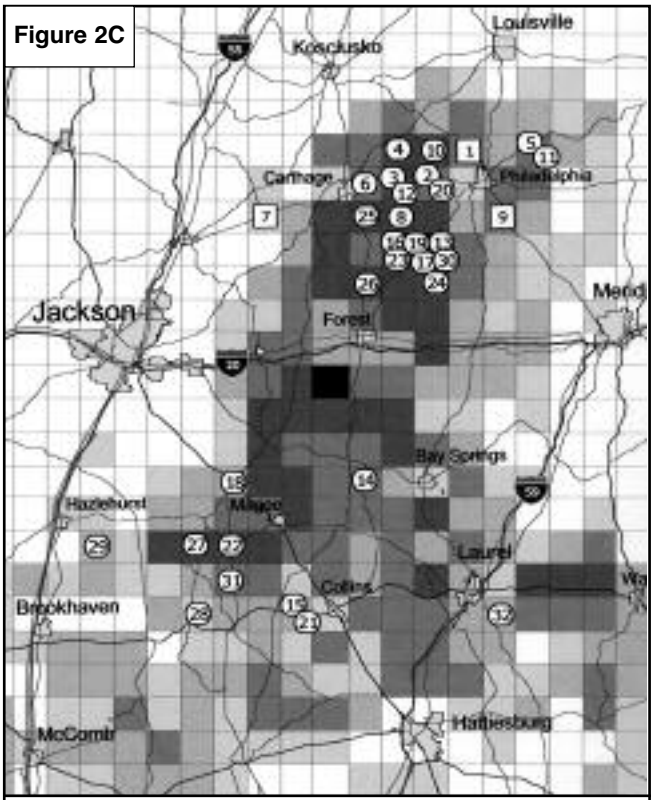
Once the episode passed, it appears that most of the industry returned to their pre-ILT level of biosecurity for both their broiler and their pullet/breeder farms. However, one company, cognizant of the periodic reports of exotic Newcastle disease (END) and avian influenza in this country, continued to maintain the heightened level of biosecurity they instituted during the ILT episode.

Figure 2B



Cumulative distribution of ILT-positive cases by the end of the first three time periods (December 11, 2002, to January 31, 2003). Note that several cases appeared outside the original vaccine zone established on January 9, 2003. (See Figure 2A for key to symbols.)

Figure 2C



Cumulative distribution of all ILT-positive cases received during the total episode (December 11, 2002, to March 17, 2003). (See Figure 2A for key to symbols.)

DISCUSSION

The recent trend in the United States is that clinical ILT episodes of this type are considered to be of MLV vaccine origin. There is considerable pressure for this position as non-vaccine-related ILT can potentially restrict exports of poultry to certain countries. In the episode reported in this bulletin, there was substantial evidence to support the theory that it, too, was vaccine-related. First of all, MLV TCO virus was present in the state. It was being used on all replacement broiler-breeder flocks at the time of the episode and had been for a couple of years. Additional support for this theory was that (1) the lesions associated with the disease were fairly mild and chronic in nature, and (2) the index case flock was ILT-vaccinated broiler-breeders.

If, in fact, the vaccine virus was the culprit, the most obvious source was the birds on the index farm themselves. The latency of herpesviridae—the group to which ILT belongs—is a well-documented phenomenon (5). Latency is the ability of virions to be carried in the host in a quiescent, noninfectious state. Then at some later point, latent virions can become reactivated—as a result of the waning immunity and sufficient stress—and released as infectious virions. Several authors have examined ILT for this ability and have had no difficulty in demonstrating viable virus for extended periods, some up to 16 months after the initial infection (2,7,13,16,23).

While it is conceivable that the virus that emerged on the index farm originated from the vaccine these birds had been given, there are several reasons to doubt this. First, the vaccine those birds had been given was the TCO product, which is generally considered incapable of producing clinical ILT. Second, these birds had been given the vaccine when they were 70 days of age, almost 1 year before they were diagnosed with ILT. A more likely possibility is that a more active form of ILT virus could have been introduced onto the index farm by company personnel or others (see below) who could have tracked the virus from another recently vaccinated farm. However, this does not explain why other companies, also having used the vaccine in their replacement breeders, did not have the problem.

To answer that question it would be helpful to consider several circumstances that came to light during follow-up investigations of the index farm—circumstances that could have increased that farm's risk to ILT. Some months before the index farm was diagnosed

Cytology: A Faster Technique for Diagnosing ILT?

Of the two techniques used to diagnose ILT during this episode—histology alone and VI-plus (virus isolation coupled with histology and/or FA)—histology alone appears to have detected the highest number of ILT-positive cases. However, histology does have some drawbacks. It is a rather time-consuming and technically demanding technique. It involves collecting whole tissues and placing them in a chemical fixative. Following fixation, the tissues are trimmed, serially dehydrated in a variety of chemical solutions, and then placed in an embedding material. This step generally requires approximately 12 hours. Next, the tissues are sliced into ultra-thin sections using a special cutter (microtome) and those sections mounted onto microscope slides. This is followed by a series of steps that chemically remove the embedding material and stain the tissue section. Under normal conditions, the entire histology procedure requires a minimum of approximately 15 hours of preparation before the material is ready to be viewed by a pathologist.

Cytology, on the other hand, can be completed in a matter of several minutes after receiving the tissues. It can be done using conventional staining techniques found in most diagnostic laboratories and requires no special equipment. To our knowledge, the use of cytology for the demonstration of either syncytial cells or intranuclear inclusion bodies associated with ILT has not been previously reported.

Cytology is the technique of obtaining fresh cells and staining and viewing them with a light microscope. Basically, the steps we used involved making impression smears by smearing the trachea mucosa directly onto microscope slides. The smears were then air-dried and dipped briefly into methanol for fixation. Next, the slides were processed through Wright-/Giemsa-like stain (Hema 3 Stat Pack, Fisher Scientific, Pittsburgh, Pennsylvania) using the manufacturer's directions. Afterwards, the slides were washed with tap water, allowed to air dry, and examined using routine light microscopy.

In our experience, the identification of the definitive intranuclear inclusion bodies associated with ILT via cytology was disappointing and did not appear to serve as a reliable method for detecting the disease. On the other hand, the presence of definitive syncytial cells appeared to be a more consistent and reliable indicator on which to base a positive diagnosis.

Twenty-six cases (including some that were not case-definition cases) were evaluated by histology and cytology. Both techniques were run and evaluated independently of each other by different individuals. Specific results are given in the table.

See Cytology on Following Page . . .

with ILT, another breeder farm under contract to the same company had labor problems. The owner abandoned the farm with breeders in production. The company resolved the situation by removing the 6,000 breeders on that farm and moving them onto three other company-contracted farms with birds of similar age. On September 22, 2002, approximately 3,000 breeders from that farm were relocated to the index farm. (The remaining birds were split between two other breeder farms. All birds on the second farm were sold before the index case broke. The third farm actually had an empty house into which the birds were moved. According to company personnel, neither of these other two farms experienced any subsequent problems.) Another set of circumstances to note is that a third breeder house was being constructed alongside the other two on the index farm at the time the birds experienced ILT. During construction, the contractor's crews entered the existing houses to connect to their water and electrical services. It is possible that one of those individuals—or others who later installed poultry equipment—could have been on another farm where the vaccine had been recently used and then mechanically transferred the virus elsewhere. Still, another situation to consider is that toward the end of November, some 2 to 3 weeks before any problem was noted, a relative of the grower of the index farm became ill and died shortly thereafter. During that period the grower was away from the farm repeatedly and depended on substitutes for daily care of the birds.

Although it was never possible to determine the precise role of any of these circumstances in initiating the episode, they do suggest possible scenarios for entry of the virus. They also indicate more than a modicum of stress for the birds on the index farm. Stress is well documented as a means of causing reactivation of latent herpes viruses in general (5) and the virus of ILT in particular (12,13). In addition to the situations already mentioned, the index case farm had two other stressors of note. *Pasteurella multocida*, a well-documented primary pathogen of poultry (20), was isolated from these birds. Incidentally, during depopulation, several pox-like lesions were noted on the shanks of some birds, indicating a concurrent or previous exposure to that virus. (No attempt was made to confirm the pox virus.)

Although our working theory is that the episode started on the index farm, there is the possibility that ILT was present and causing other problems in the state

. . . Cytology

Twenty-six ILT-suspect cases evaluated by histology and cytology.

ILT diagnosis by		Cases
Histology	**Cytology**	
positive	positive	14
positive	negative	3
negative	positive	0
negative	negative	9
Total		**26**

As can be seen, there was complete agreement between histology and cytology—either both positive or both negative—for 23 of the 26 (88%) cases evaluated. Of the 3 remaining cases (12%), histology detected all of those to be ILT positive but cytology did not.

When comparing the efficacy of a "new" test such as cytology against a more traditionally accepted, "gold standard" test such as histology, it is customary to plot the values in a 2-by-2 contingency matrix as follows:

	gold standard +	***gold standard –***	
new test +	**a**	**b**	**a + b**
new test –	**c**	**d**	**c + d**
	a + c	**b + d**	

Once this is done, the *sensitivity* or ability of the new test to accurately detect true positives can be calculated using the formula: **a / (a + c)**. The *specificity* or the ability of the test to detect true negatives can be calculated using the formula: **d / (b + d)**.

By inserting the values from the table into this matrix, we found that the calculated sensitivity of cytology was 82.4% and the calculated specificity 100%:

	histology +	**histology –**	
cytology +	**14**	**0**	**14**
cytology –	**3**	**9**	**12**
	17	**9**	

The same data can be used to calculate the predictive values of the test—the probability that a flock is ILT+ if the cytology is positive (known as the positive predictive value or PVP) or the probability that a flock is ILT– if the cytology is negative (known as negative predictive value or PVN). These are calculated by the formulae: **PVP = a / (a + b)** and **PVN = d / (c + d)**. Based on these data, the PVP and PVN are 100% and 75%, respectively.

In conclusion, based on this limited set of data, it appears that cytology shows promise and is worth continual evaluation in a larger number of cases and/or in a series of controlled experiments. In the meantime, cytology can provide a useful screening method for providing a rapid presumptive diagnosis for the presence of the ILT virus.

prior to its detection on the index farm. After the episode was over, rumors surfaced that broiler farms in the vicinity of the index farm had experienced increased mortality associated with a respiratory condition shortly before the index farm broke. However, conversations with the responsible parties never confirmed this nor were any birds with this type of history submitted for laboratory examination.

Regardless of the source of the initiating virus, once it was established, it most probably spread by mechanical transmission by a variety of means: company personnel, utility and delivery individuals that had business on the farm, and possibly, the growers themselves. All of these have been well documented in the past (3).

It is difficult to evaluate the overall level of biosecurity exercised by the state's poultry industry at any point in time, but given the relatively low level of disease in the state, it is generally agreed that it was fairly lax at the time this episode occurred. That being the case, it is surprising that ILT did not spread over the entire state. Once the episode had spread past the initial vaccine zone (after January 8, 2003) efforts to heighten the awareness of the disease and biosecurity measures necessary to combat it were redoubled. In addition to the industry and the growers, these efforts were also extended to those in satellite industries that supported poultry (e.g., vaccine and drug representatives, utility providers, etc.).

The possible role of other, non-ILT, microbiological agents as stressors in the overall episode could not be determined. Concomitant infections with known stressors, such as infectious bronchitis and Newcastle disease viruses, together with the presence of potential stressors, such as *B. avium* and *O. rhinotracheale*, were detected throughout the cases received. With few exceptions, their numbers were fairly evenly distributed between ILT-positive and ILT-negative cases. Consequently, they do not seem to have participated as a cofactor in the episode.

This episode reinforces the need for diagnosticians to be constantly alert to the varieties of infectious diseases and their range of expression. This is particularly important given the increased occurrence of avian influenza and exotic Newcastle disease (END), and the general threat posed by bioterrorism. Speculation among the authors is that the index case could have been easily dismissed as a severe pneumonia because that was the most pronounced lesion observed. Tracheal lesions, the more pathognomonic indicator of more acute forms of ILT, were seen only in dead birds and could easily have been dismissed as postmortem artifacts. The nature of the lesions seen in the index farm chickens is commonly seen on necropsy and is generally attributed to other (non-ILT) causes. Consequently, tissues are not always collected for histopathological or microbiological examination from lesions of this type. Furthermore, the authors noted that if the index case had not been diagnosed as ILT and heightened their awareness, the initial ILT-positive broiler cases might have been overlooked too and dismissed as respiratory consequences of heightened ammonia levels.

COMMENTS

Because there was no documentation of the 1981-82 episode, personnel involved with the 2002-03 episode had to relearn some old lessons. They are listed here for those who have to deal with future episodes of this disease. Noteworthy also is the article by Bagust and Johnson (4) in which the theoretical and practical aspects of ILT prevention and control are reviewed.

Biosecurity

The importance of biosecurity in preventing or controlling this and other infectious diseases cannot be stressed enough. Unfortunately, however, "biosecurity" seems to register in the minds of some poultry company personnel and their growers as a negative factor because it generally requires acquiring additional equipment (disinfectant sprayers, wash stations, etc.), consuming supplies (disinfectant, boots, gloves, etc.), and requiring additional time to conduct the necessary procedures. The intangible aspect of biosecurity and the lack of any positive reinforcement received for doing it are equally unfortunate. That is, when it works, nothing (untoward) happens!

For these reasons, the tendency typically is to exercise no more biosecurity measures than are necessary to meet the threat at hand. Given the additional output required, this might make economic sense in the short run, but the effect infectious diseases have on profitability and the potential effect they have on exports more than compensate for this effort. Continual investments in equipment, supplies, and training would also place the industry in a better position once a threat is present, rather than waiting until the threat occurs. Furthermore, the heightened awareness that accompanies more rigorous biosecurity efforts should have the added benefits of increased vigilance for diseases and gearing the industry more toward prevention, which is less costly in the long run than treatment.

As far as ILT is concerned, the main points to remember when considering biosecurity measures are (1) the persistent nature of the virus outside the host, and (2) that the virus can be spread directly by aerosol and indirectly by viral-contaminated objects (3,11). Care should be taken to disinfect vehicles, including floorboards; to change clothing; and to use shoe covers or disinfected boots. Also during this episode, there was a concerted effort to curtail routine company and grower meetings and to handle communications more indirectly by phone and/or by electronic means.

In retrospect, there are several additional biosecurity measures that could have been implemented to facilitate the control of ILT in this episode. The drifting of feathers and other detritus from live-haul trucks is considered an important means of transmitting the virus (3). Therefore, corridors for transporting infected and CEO-vaccinated birds to their respective processing plants should be established by mutual agreement of the poultry companies. The major consideration in establishing these routes are to locate routes that pass the least number of poultry farms. Other measures could have been implemented as well (e.g., restrictions on spreader trucks, live-haul trucks, use of shared equipment, etc.).

Communication and Cooperation

We suspect that one of the attributes that separates Mississippi from some other poultry-raising states is the openness with which the state's poultry industry approached this episode. The considerable degree of communication that occurred among the state's commercial companies and the regulatory and diagnostic agencies was key to accurately identifying the cases in this disease and to controlling it in a fairly brief period. The commercial companies and the regulatory and diagnostic agencies all worked together to formulate policies and procedures that were practical and could be followed throughout the episode. A prime example was the agreement to vaccinate all flocks within the state and to start and stop vaccinating on specifically agreed-upon dates.

Surveillance

One of the first steps that should occur when ILT is suspected is to increase surveillance. It is paramount that all cases of respiratory distress, especially those not associated with postvaccination respiratory reactions, be submitted promptly for laboratory analysis. This is particularly important for poultry grown in proximity to other ILT-positive or other ILT CEO-vaccinated poultry. This disease episode had a relatively short lifespan. However, if the respiratory problems rumored to have been in broilers prior to the index case did occur, and if those birds had been submitted and had been found to be ILT positive, this episode might have been shortened even more. More importantly, it might have permitted containment in a small vaccina-

tion zone and eliminated the need to require all broilers in the state to undergo CEO vaccination and the negative consequences that entailed.

There was one omission in the surveillance of this episode that needs mentioning. "Backyard" birds are considered potential hosts for the ILT virus (4). However, given the limitations of manpower available for surveillance and the lack of knowledge on the particulars—locations, numbers, etc.—of birds in this category, no effort was made to monitor them for the disease.

Diagnostic Tests

Of the laboratory techniques that were used to diagnose ILT, VI coupled with histological examination and/or fluorescent antibody testing of suspect CAM lesions (VI-plus) is recognized as a "gold standard" by which other tests are evaluated (1). However, in this particular episode, direct histological examination of specific tissues—trachea, lung, and lower eyelid—and the finding of intranuclear inclusions in freshly collected necropsy samples detected 30 of the 31 ILT-positive cases evaluated by both techniques (direct histology and VI-plus), while VI-plus detected only 25 of the 31 ILT-positive cases. This is somewhat contrary to the published literature, which indicates that direct histological examination of necropsy tissues is a poor substitute for VI and other techniques (1). One major difference between the two techniques is the time to completion. Histological examination of necropsy tissues can be completed within a day or so of receiving the tissues, whereas VI-plus requires a minimum of 10 days. Despite the additional time required, however, VI procedures should still be run on all submitted cases as the end product is a supply of live virions, which can be used, if needed, to conduct subsequent *in vitro* or *in vivo* studies.

Handling ILT-Positive Farms

Once ILT is diagnosed on a farm, there are several measures that should be taken. The premises should be quarantined and every effort made to enforce it. Access to the farm should be limited to only essential personnel and services, and these individuals and their vehicles should adhere to rigorous biosecurity measures to prevent the virus from leaving the premises. Specifically, anyone having to enter a poultry house, including especially the grower, should make ample use of disinfectants and protective clothing and footwear. As much as possible, the grower and his/her family should avoid other growers and their family members. As ILT is not known to be transmitted *in ovo* (3), eggs from infected breeders can continue to be collected, provided that they are cleaned free of feathers and feces and that the flats used to transport them to the hatchery are thoroughly cleaned and disinfected before reusing. However, because ILT is known to be transmitted by fomites (14), every effort should be made to eliminate possible transmission via contamination on equipment and supplies used in conjunction with hatching operations. In order to prevent the potential spread of the ILT virus, ILT-positive breeders should remain on the farm for at least 4 weeks after diagnosis, even if that is past the point when the flock is due to be slaughtered.

It is deemed very useful to have a protocol in place for handling ILT-positive farms once the birds have been removed. This applies to both pullet/breeder farms and broiler farms that have been diagnosed as ILT positive and to those farms on which CEO ILT vaccine was used. The litter should be wind-rowed down the center of the house to facilitate composting. Heat should be elevated in the house to 100°F for a period of 100 hours to assist with the composting. Curtains should be up and fans turned off during that time. Heat-treated houses should be allowed to remain free of replacement birds ("down-time") for a minimum of 21 days after the infected birds are removed.

Despite these recommendations, the practical reality is that the episode occurred during the cooler months of the year when the highest temperature that could be attained in most chicken houses was 80°F to 85°F. Another practical reality is that economic pressures tended to decrease the 21 days that were recommended as down-time to rest the house before placing the next flock.

In retrospect, the killing and burying of the birds on the index farm in this episode was probably unnecessary considering that no other company burdened itself with depopulating its ILT-positive farms and still the episode was resolved in a relatively short period (about 3 months).

Use of MLV Vaccines

The decision to use MLV ILT vaccines should not be taken lightly as the viruses in these vaccines, especially those in CEO products, are considered potentially responsible for keeping aspects of the disease present (4). Furthermore, the consensus of those involved in

this current episode believed that the cost of the cure was higher than the cost of the disease. ("Cure" being the cost of the vaccine and its application and the loss of productivity due to the morbidity that accompanied vaccine-related reactions.)

Once the decision is made to vaccinate with CEO ILT vaccines, and to vaccinate with a less-than-statewide approach, there are several concepts to keep in mind in determining a vaccine zone.

Defining the location to vaccinate. Technologies such as GPS coupled with GIS databases can be used to determine geographic attributes important to establishing a vaccine zone. This includes the location of poultry farms, numbers of birds, and number of poultry companies present. Once a serious disease threatens, these technologies can be used to design the borders of a zone using "natural" separations in the distribution of poultry farms.

Size of the zone. Vaccine zones should not be too tightly constructed around known ILT-positive farms, but they should be large enough to allow some additional "buffering" territory around the known positive farms. Many felt that the 1-mile zones used around infected premises in 1981-82 were too restrictive, hence the use of the larger zone attempted in 2002-03.

Live-haul corridors. Once established, poultry farms located in close proximity to these routes or those having access lanes that connect to these routes should be considered in the vaccine zone and vaccinated as well.

Total agreement and cooperation of participants. It is essential that all companies within a designated vaccine zone agree to use the vaccine. Equally important is that they also agree to the same starting and stopping dates and continually vaccinate between the two. The industry's adherence to these guidelines should contribute to the rapidness with which episodes of this type are controlled in the state.

Type of vaccine and method of application. The TCO ILT vaccine was restricted to breeder replacements. It was given by the eye-drop method when the birds were about 70 days of age and considered fairly innocuous.

The use of CEO ILT vaccine is a more complicated issue. It is widely agreed that CEO vaccines produce more pronounced respiratory reactions in the recipients than do TCO vaccines. A minimal amount of reaction is generally welcomed as an indication that the vaccine is "working." However, too much reaction is considered undesirable as it has the potential to lead to more pronounced respiratory disease when mixed with other agents in the chicken house, such as other MLV vaccine viruses, *E. coli*, dust, and ammonia. Another concern is the fact that the viruses in CEO ILT vaccines have been shown to increase in virulence after being passed from bird to bird (9), which could happen naturally during "rolling" reactions in a chicken house.

During this episode, CEO ILT vaccines were permitted as a secondary ("boost") vaccination for pullet/breeders. However, this practice was strongly discouraged due to concerns about "seeding down" these farms with the virus. Unlike broilers, broiler-breeders remain on their farms for extended periods providing a favorable environment for the virus's survival. This allows them to serve as carrier birds (i.e., a source of other infections). Indications are that some companies used CEO ILT in their pullet/breeder flocks during this episode. Observations are that, while the vaccine did cause some severe reactions, no clinical ILT was detected (personal observation, Dr. Phil Stayer).

In broilers, CEO vaccine had some consequences of note, too. When the vaccine was initially given via the drinking-water method, several people reported untoward reactions in CEO vaccinates including harsh and continual ("rolling") reactions some of which were associated with substantial mortality. Some of these reactions were undoubtedly caused by people who had no previous experience applying the vaccine and no knowledge of its various routes of administration. As the episode progressed, many companies found that these reactions could be ameliorated by giving the vaccine by spray application. On an equivalent dose basis, vaccine given by spray was found to produce less severe reactions compared with drinking water vaccination. However, spray vaccination still produced a small degree of rolling reactions. Subsequently, it was found that diluting the vaccine to a half dose per bird seemed to help with the severity of the reactions. (It should be mentioned that the lack of any detectable reaction could be unsettling to the growers and their company supervisors.) The reduced-dose vaccine might increase mortality slightly for 5 to 6 days after spray vaccination, but it would subside quickly. Overall, the feeling is that the lower dose of vaccine given by spray was a good balance between the degree of reaction and providing adequate protection. Some broilers developed severe "glaucoma" after LT vaccination when the vaccine was given via spray.

"Dangerous contact" flocks. Flocks that are near an infected flock should be included in the vaccination zone. This can be defined by things such as geography, distance, travel routes, and prevailing winds. However, some flocks are dangerous contacts because of the connections of people associated with the farms: relatives, roommates, and close social contacts.

Statewide vaccination should be used only as a last resort, as it was here when it was determined that new cases were appearing well beyond the established vaccine zone. In retrospect, it may have been possible to have avoided this by enlarging the existing zone to encompass the cases in the northern cluster and by establishing a second vaccine zone for the southern cluster of cases. However, that opinion was formed in hindsight after seeing the total extent of ILT-positive cases once the episode has passed. During the episode, the intensity of both the number of new positive cases and their dispersed locations prompted the decision to go with statewide vaccination.

The downside to blanketing the state with CEO vaccine is the potential that that virus could linger on poultry premises and cause persistent cases of the disease. One of the reasons given for why Mississippi has so little ILT and has been able to minimize the extent of its ILT episodes is because of not permitting the use of live ILT vaccines. Thus, the commercial poultry houses, and possibly the backyard flocks, in the state are not seeded down with the virus. Circumstantial proof for this is the intermittent prevalence of ILT in the Southeast (21), which was not detected in Mississippi, and the several occurrences in adjacent Alabama (personal communication with poultry contacts in Alabama), which did not spread into Mississippi. It is possible that the conservative use of CEO vaccine in Mississippi allowed the state to be more disease-free while other states were not.

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Appendix A. Responses to questionnaire sent to commercial Mississippi **pullet/breeder** operators about their biosecurity practices. Responses are listed in three columns: 1) during the 2002/03 infectious laryngotracheitis (ILT) episode; 2) Was that practice a change from the level of biosecurity practiced the outbreak occurred? and, if a change (indicated by number in bold box), 3) Was that biosecurity practice continuing (after the outbreak)?

Pullet/breeder operations [10 questionnaires sent, 8 returned, 7 respondents][A]	1) During outbreak		2) Is this a change from before outbreak?		3) Did this practice continue after outbreak?	
	yes	no	yes	no	yes	no
Service personnel						
Were their routes altered in any way?	2	5				2/2
• CEO- vaccinated pullets visited last • went from youngest to oldest flocks						
Was the frequency of visiting farms altered?	2	5				2/2
• less frequently • limited to only necessary trips						
Were they instructed not to go to other farms once they encountered a respiratory-diseased farm?	7	0	3	4	?/3	2/3
• until verified, consider respiratory disease as ILT • take birds to lab, go home, clean up, and disinfect • if they had a farm to break, minimize the visits to this farm and when they did visit, make it their last stop and completely disinfect vehicle and change clothes • go home, shower, change clothes, and footwear before returning to work • complete clean-up of personnel and vehicle						
Were they discouraged from participating in any non-company activities?	2	5	2	5		2/2
• stop all unnecessary contact • discouraged all such activities						
Were any special measures taken to insure footwear protection/disinfection before entering a chicken house?	7	0	1	6		1/1
• use disposable boots before entering a poultry house [2 respondents] • plastic boots required on all farms plus foot baths on some farms • boots and foot pans • foot boots						
Were any special measures taken to insure footwear protection/disinfection after visiting a chicken house?	7	0	3	4	2/3	1/3
• disinfect foot-wear before leaving farm • disinfect boots; aerosol spray disinfectant on floor mats of truck • plastic boots required on all farms plus foot baths on some farms • disposable boots • foot boots						
Was the wearing of coveralls or over-clothing required when visiting chicken farms?	7	0	0	7		
• always been our standard [5 respondents] • always stressed to use fresh clothing • disposable scrubs						
Were any of your pullet/breeder flocks vaccinated with CEO/egg-propagated (in addition to TCO or tissue-culture propagated) vaccine? (If yes, were there any special instructions for visiting these and non-CEO-vaccinated flocks?)	5	0				
• eliminate contact between vaccinated pullets and non-vaccinated birds • visit CEO-vaccinated farm last thing of the day [2 respondents] • always visit CEO farm last until it is TCO-vaccinated • don't visit vaccinated birds before non-vaccinated birds						

Other than what's been asked above, were any other routine changes put into effect?

• disinfect vehicles before and after trips to farm • no outside visitors

Feed mill deliveries

Did any of their activities change once it was known that LT was in the state?

• disinfect tires, etc. • all vehicles disinfected before and after deliveries • disinfect all incoming trucks • sprayed vehicle wheels upon entering and leaving farms; increased washing of vehicles more frequently • feed trucks were disinfected and protective footwear was used by driver; trucks did not go from infected to uninfected farms • truck disinfectant station at mill and foot protection on farms

Non-company (installation, repair, utility, construction, etc.) personnel

Did any of their activities change once it was known that LT was in the state?

• all required to wear protective foot wear and not to enter houses unless absolutely necessary • asked vendors to wear protective footwear while on farms and disposable coverall if they had to enter a house • grower supplied these individuals with disposable boots for use before entering house • they were made aware of the seriousness of the ILT threat; told to use boots, etc. • require suppliers to wear protective gear and disinfect vehicles • disposable coveralls and boots

Spent-hen catchers

Did any of their activities change once it was known that LT was in the state?

• increased their awareness of biosecurity concerns • notified MBAH of travel routes to plant

Growers

Were any special measures taken to ensure footwear protection/disinfection?

• instructed them on proper use of foot baths • used foot baths at farm entrances • kept fresh disinfectant and changed it daily • asked to use footwear for chicken houses only • foot baths • boots and disinfectant

Was the wearing of coveralls or over-clothing required?

• asked to use clothes for chicken houses only • always required

What were they instructed to do with dead birds?

• prompt and proper disposal [7 respondents]

What were they instructed to do when they suspected the birds had a respiratory problem?

• call service tech/main office as soon as possible [3 respondents] • call service tech; faster response time after receiving grower's call • call service person and report accurate mortality for the last 3 days

Were they discouraged from having visitors on the premises?

• discouraged all visitors [2 respondents] • asked growers to allow only necessary visitors on farm • informative letter sent out • discourage all visitors

2	5				2/2
6	1			2/6	4/6
6	0[b]			2/6	4/6
2	5			1/2	1/2
7	0	1	6		1/1
3	4	1	6		1/1
		0	7		
		5	2	1/5	4/5
7	0		7		

Were they discouraged from visiting other poultry premises?	7	0	1	6	1/1	
• told not to visit other farms [2 respondents] • asked not to visit other farms • don't allow • informative letter sent out						
Were they discouraged from any off-the-farm activities?	7	0	4	3		4/4
• when going to community stores, etc., change clothes and shoes before going back to the chicken house • informative letter sent out • made aware of possible transmission • asked growers to limit exposure to other growers at store • discouraged off-farm grower meetings • told not to frequent stores, meeting places, etc.						
What was the average or range of "down-time" between flocks?			0	7		
• >2 wks for pullets; > 6 wks for hens • 2 to 3 wks for pullets; 6 to 7 wks for hens • 2 to 3 wks for pullets; 4 to 6 wks for hens • 3 to 5 wks pullets and hens • 4 to 6 wks • 1 mo • 2 mo						
Was anything done to the litter in the house between flocks?	5	2	1	6		1/1
• some pullet houses cleaned-out, otherwise, "cake-out;" hen houses cleaned-out and disinfected • hens cleaned out and litter spread not stockpiled • pullets acc breeders clean-out • windrowed and heated between flocks • clean-out						
Was any equipment that was used on one premises used on another premises?	5	1[B]	0	6		
• in some instances, this happened at clean-out; asked growers to make sure equipment was properly disinfected before and after use • disinfect catch pens, etc. • clean and disinfect • service personnel double checked on disinfectant procedures • growers told not to use any equipment unless it was disinfected first						
Additional/miscellaneous comments						
Other than what has been covered above, did any other company activities change once it was known that LT was in the state? (Or are there any other comments you would like to add?)	4	3				4/4
• discouraged all grower and company personnel from gathering of any kind • disallow all visitors • increased vigilance on vehicle cleanliness • restricted outside visitors						

[A]One of eight responses returned had no pullet/breeders in their operation.

[B]One respondent unsure of this.

Appendix B. Responses to questionnaire sent to commercial Mississippi **broiler** operators about their biosecurity practices. Responses are listed in three columns: 1) during the 2002/03 infectious laryngotracheitis (ILT) episode; 2) Was that practice a change from the level of biosecurity practiced before the outbreak occurred? and, if a change (indicated by number in bold box), 3) Was that biosecurity practice continuing (after the outbreak)?

Broiler operations [10 questionnaires sent, 8 returned, 8 respondents]	1) During outbreak		2) Is this a change from before outbreak?		3) Did this practice continue after outbreak?	
	yes	no	yes	no	yes	no
Service personnel						
Were their routes altered in any way?	4	4			1/4	3/4
• youngest to oldest birds • any suspect farms visited last thing for the day • techs servicing only clean or dirty areas, not both • worked in and around vaccination						
Was the frequency of visiting farms altered?	3	5				3/3
• during the "hot" break time, used the phone; visited only by call, then went home • more time required for biosecurity steps and vaccinating • avoid farms in possible "hot" areas and limited activities on farms in areas with other companies						
Were they instructed not to go to other farms once they encountered a respiratory-diseased farm?	7	1	5	3	2/5	3/5
• complete clean-up of personnel and vehicle (including disinfection) [2 respondents] • until verified, consider respiratory disease as ILT • take birds to laboratory, go home, clean-up, and disinfect • follow MPA ERAD outline protocol[3] • notify management, go home, take bath, change clothes, and wash vehicle before entering another farm • shower and wash truck; service by phone						
Were they discouraged from participating in any non-company activities?	5	3	5	3	2/5	3/5
• stop all unnecessary contact • service personnel and growers asked not to congregate with other poultry people or at "poultry places" • restrict activities that involved other poultry or other company personnel • no exposure to those associated with other poultry • limit traffic where growers congregate						
Were any special measures taken to insure footwear protection/disinfection before entering a chicken house?	7	1	4	4	2/4	2/4
• disposable boots [4 respondents] • plastic boots plus spray-on disinfectant • rubber foot-ware • disposable boots and foot baths						
Were any special measures taken to insure footwear protection/disinfection after visiting a chicken house?	8	0	1	7		1/1
• dispose of boots on farm [3 respondents] • boots worn from and to vehicle • spray shoes and floor of pickup • disposable boots • rubber foot-ware						
Was the wearing of coveralls or over-clothing required when visiting chicken farms?	2	6	2	6	1/2	1/2
• hospital scrubs and head-ware • disposable coveralls						

During the two "transition periods" (1) time leading into and, 2) time leading out of the state-wide vaccination effort when some broilers were vaccinated and others not), were there any special instructions or restrictions for visiting LT-vaccinated and non-LT-vaccinated flocks?	7	1[B]		
• went to non-vaccinated first, then to vaccinated [4 respondents] • made "phone visits" to any suspect farms • after birds were vaccinated, no service visits unless grower called, then go home				
Other than what's been asked above, were any other routine changes put into effect?	5	3	2/5	3/5
• daily updates of suspects farms of all companies • so much time spent vaccinating that other tasks were left undone • developed preparedness kit so service personnel would have everything they needed to take birds and/or tissues if they encountered a suspect flock and to ensure containment • all vehicles were sprayed with disinfectant before leaving growers, office, or processing plant • controlling litter to and from farms				
Feed mill deliveries				
Did any of their activities change once it was known that LT was in the state?	7	1	1/7	6/7
• disinfect trucks and tires • total disinfection of all vehicles before and after deliveries • disinfect all incoming trucks • disinfect tires on vaccinated farms • feed trucks were disinfected and protective footwear was used by driver • truck wash/disinfectant station at mill and foot protection on farms • cautioned drivers to disinfect tires when possible				
Hatchery				
Did any of their activities change once it was known that LT was in the state?	7	1	4/7	3/7
• disinfected chick boxes and equipment before leaving farm and as leaving state • started foot-baths, disinfected wheels, and disinfected foot-wear • provided scrubs and outer covering for employees • disallow all visitors • truck disinfectant station and foot protection on farms • total disinfection of all vehicles before and after deliveries • driver restricted from entering houses				
Catching crews				
Did any of their activities change once it was known that LT was in the state?	2	6	2/2	
• clean vehicle as much as possible before leaving farms; change routes when possible [2 respondents]				
Live-haul trucks and drivers				
Did any of their activities change once it was known that LT was in the state?	3	5	2/3	1/3
• disinfect trucks going to farms • all trucks were sprayed with disinfectant before entering and after leaving farm • when possible, disinfected all catching vehicles and changed routes				
Non-company (installation, repair, utility, construction, etc.) personnel				
Did any of their activities change once it was known that LT was in the state?	7	0[C]	4/7	3/7
• required suppliers to wear protective gear and disinfect their vehicles [2 respondents] • grower supplied these individuals with disposable boots for use before entering house • asked vendors to wear protective footwear while				

on farms and not to enter houses; asked power company to estimate usage during this period • contacted all suppliers and vendors and asked them to use footwear and disinfect their vehicles • required to use plastic boots or disinfected rubber boots • foot baths at front doors; wore foot bags

Growers

Were any special measures taken to ensure footwear protection/disinfection?

• required them to have foot baths; asked them to change footwear and clothes before and after returning home from visits • instructed by service personnel on use of foot baths • asked to use footwear that could be restricted to chicken houses only • required on suspect farms

Was the wearing of coveralls or over-clothing required?

What were they instructed to do with dead birds?

• prompt and proper disposal as usual [8 respondents]

What were they instructed to do when they suspected the birds had a respiratory problem?

• call service tech/main office [6 respondents] • call service tech (faster response time expected from tech after receiving grower's call) • call service person and report accurate mortality for the last 3 days

Were they discouraged from having visitors on the premises?

• no admittance [3 respondents] • discouraged all visitors [2 respondents] • asked growers to allow only necessary visitors on farm • informative letter distributed • only necessary personnel

Were they discouraged from visiting other poultry premises?

• told not to visit other farms [4 respondents] • importance of disease was explained to growers so they understood the dangers [3 respondents] • kept growers undated

Were they discouraged from any off-the-farm activities?

• made aware of possible transmission risk this posed [3 respondents] • asked to limit these activities as much as possible and to change clothing if they did participate • change clothes and foot-wear before going back to the chicken house • asked growers to limit exposure to other growers at store • discouraged off-farm grower meetings • only activities involving other poultry personnel

What was the average or range of "down-time" between flocks?

• 7 days • 8 to 10 days • 10 to 14 days [3 respondents] • 14 days • 21 days • ≥ 3 wks

Was anything done to the litter in the house between flocks?

• piled litter inside house and allowed to go through a heating period (brooders on for 24 hrs) • on suspect or positive farms rowed, composted, heated • composted, heated house to 100 degrees for 100 hrs • rolled and heated after vaccinating last flock • row-up letter; heated house to 100 degrees for 48 hrs • windrowed litter, raised curtains and increased heat

4	4	2	6	1/2	1/2
0	8	0	8		
		0	8		
		3	5	1/3	2/3
8	0	3	5	2/3	1/3
8	0	2	6	1/2	1/2
7	1	4	3	2/4	2/4
		1	7	1/1	
6	2	6	2	2/6	4/6

Was any equipment that was used on one premises used on another premises? • permitted but not encouraged • catching equipment, vaccinating pump • clean and disinfect • not allowed unless disinfected [Note: 3 respondents indicated that this practice was stopped during the outbreak period]	4	3[C]	4	3	2/4	2/4
Additional/miscellaneous comments						
Other than what has been covered above, did any other company activities change once it was known that LT was in the state? (Or are there any other comments you would like to add?) • discouraged all grower and company personnel from gathering of any kind • disallow all visitors • service vehicles were disinfected before and after entering a farm • restricted outside visitors • company went to ERAD Level 2[A] • cancelled monthly grower meetings at the hatchery	5	3			2/5	3/5

[A]See Appendix D.

[B]One respondent did not use CEO vaccine in broilers.

[C]Respondent unsure about this.

Appendix C. Responses to questions about the effectiveness/ineffectiveness of the how the 2002/03 ILT episode was handled.

In your opinion, what was done correctly to diagnose, control, and eradicate the problem? [10 questionnaires sent, 8 returns, 5 respondents]

1. • Early awareness and admitting that LT in the state.
   • Commitment from diagnosticians to work long hours for speedy information.
   • Sharing laboratory and field vaccine results.
   • Broad geography of vaccinating.
2. • Strict biosecurity and everyone staying informed and working together.
3. • Proper procedures were done with communication from day of diagnosing: birds were taken to lab and then communicated back to management whereupon directions were given to the grower.
   • A good job administering the vaccine.
   • We were very open from the onset of the problem and did not try to hide anything.
   • The willingness of all companies to vaccinate when we realized the spread was growing.
   • Cooperation between companies in travel routes to plants and statewide vaccination.
4. • Companies cooperated very well and, as always, followed procedures as outlined by the Mississippi Board of Animal Health.
   • Once the reality of an outbreak occurred in a community, the growers responded in a very positive manner.
   • Communications between companies and all state agencies worked well.
5. • Quick response from state veterinarians and laboratories.

In your opinion, what could have been done to improve the diagnosis, control, and eradication of the problem? [10 questionnaires sent, 8 returns, 4 respondents]

1. • Start spray vaccinating earlier
   • Vaccinate corridors in "hot" areas.
   • Limit vehicular traffic from north of I-20.
2. • Some companies could have been faster getting suspect birds to the laboratory
   • Complete openness from everyone from the start.
3. • Because disease may have been in the state long before the first diagnoses were made (maybe in a very mild form) and then spread to the processing and hen plants before we know it was here; these routes (to-and-from the processing plants) should have been vaccinated as soon as possible.
   • Applying a vaccinating zone was of questionable value.
   • The CEO vaccine in broilers is about the same as the disease; also, we spread the disease more with the vaccine than before the vaccination.
   • We jumped from a zone-approach to a statewide vaccination too quickly.
4. • Vaccinated entire state too quickly.
   • Too many non-state veterinarians made too many decisions.
   • Did not use control measures everyone agreed to (*i.e.*, litter movement, heating houses).

APPENDIX D

THE MISSISSIPPI PLAN:

A MANUAL FOR THE PREVENTION AND CONTROL OF EMERGENCY, REPORTABLE, AND ACTION DISEASES (ERAD) OF POULTRY

SPRING 1995

Amid increased concerns about serious poultry diseases occurring in the United States during 1994, a group of Mississippi poultry health specialists consisting of Danny Magee (McCarty Farms), Reagan Sadler (Central Industries, Inc.), Henry Welch (Peco Farms, Inc.), Georgia Brown and Danny Thornton (Mississippi Board of Animal Health), Phil Rhinewalt (Arbor Acres, Inc.), Roy Montgomery (College of Veterinary Medicine), Greg Jordan (Wayne Farms, Inc.), Tim Wals-leben (B. C. Rogers, Inc.), Steve Roney (Sanderson Farms, Inc.), and Hugh Stout (Southern Hens, Inc.) met several times during the winter of 1994/95 and produced this plan. The plan was to be part of a strategy that would enable the state's poultry industry to prevent, control, and eradicate **emergency** diseases, those which are exotic and for which Federal programs exist for their eradication (including exotic Newcastle disease, avian influenza, ornithosis, etc.); **reportable** diseases, those considered inordinately disruptive to the poultry industry; and **action diseases**, those designated by the State for immediate action to eradicate or control (such as infectious laryngotracheitis, infectious coryza, etc.). Collectively these type diseases became known as "ERAD"s.

The group gratefully acknowledge the Delmarva Poultry Industry, Inc., for permitting its *Procedure Manual on Emergency Poultry Diseases* to serve as the basis of this document.

What follows is the plan that was developed over the winter of 1994/95. It should be noted that 1) the various Biosecurity Procedures for…were intended to be on separate perforated pages so that they could be removed, copied and distributed to the intended individuals, and 2) some of the names, addresses, and phone numbers are no longer current and have been replaced.

WHAT TO DO TO PREVENT AND CONTROL ERADs – THE MISSISSIPPI PLAN

The success of an effective defensive program depends on efficient **discovery** and **reporting** of an ERAD wherever it appears. Perhaps the most critical period is the time between when the infection makes its "silent" entry and when it finally becomes recognized.

Defensive management is built around a program, which is practical and acceptable, yet still prevent introduction of an ERAD. Unwittingly "stumbling" onto a hazardous problem and tracking it from premise to premise represents the industry's greatest threat. To avoid this, a strong defense must be in place and maintained at all times.

THE MISSISSIPPI PLAN is a practical plan for preventing the introduction and spread of an ERAD throughout the state. It consists of three levels. The first of these, Green Level I, is the default level and is in effect at all times unless changed by the Mississippi Board of Animal Health to meet the threat of an ERAD. The response will be proportional to the threat at hand. **Note the levels are cumulative**. That is, when a Yellow Level II is declared, the conditions set forth for Levels I and II are both in effect, and when a Red Level III is declared, the conditions set forth for all three levels, (I, II, and III) are in effect. An overview of the three levels of *THE MISSISSIPPI PLAN*:

Green (I) **Minimal program where no known ERAD exists or is distinctly remote**

It is prudent to assume that ERADs may appear anywhere, at anytime, and to design the defense accordingly. A continuous effort is required. This would be minimal if no ERAD is reported.

Yellow (II) **Preventive program if ERAD occurs in a nearby area posing a threat to Mississippi**

The discovery and reporting of an ERAD anywhere in an area likely to pose a threat to the Mississippi poultry industry would call for an assessment and rating of that risk and the implementation of a more stringent defense.

Red (III) **A plan of action in event of an ERAD outbreak in Mississippi.**

This plan of action would be implemented in the event of an ERAD outbreak in Mississippi. Its success would depend on:

- Reaction time when ERAD is first suspected
- Readiness of personnel, phone numbers, procedures to follow, and access to needed supplies
- Inventory information to enable establishing industry quarantine
- Understanding dynamics of poultry industry; *viz.*, hauling area, market area, etc.

DEFINITION OF TERMS USED

biosecurity = procedures that embrace all precautions essential to protect animals from exposure to disease organisms

bird = any warm-blooded vertebrate of the class, *Aves*, having a body covered with feathers and forelimbs modified into wings

dealer = one who buys and sells articles without altering their condition; a trader or merchant

exotic = of foreign origin, not native, introduced from abroad

game bird = any bird hunted chiefly for sport, as a quail or pheasant, especially such a bird that is protected by game laws.

poultry = any domesticated fowl, especially those valued for their meat or eggs, such as chickens, turkeys, ducks, geese, guinea fowl, etc.

waterfowl = a water bird, especially one that swims, *e.g.*, a swan, goose, or duck

wildfowl = any non-domesticated bird

THE MISSISSIPPI PLAN: GREEN (LEVEL I)

Minimal Program Where no Known ERAD Exists or is Distinctly Remote

BASIC BIOSECURITY GUIDELINES

Due to reliance on vaccination and medication as its primary disease control strategy—and less reliance on sanitation and security—the poultry industry is more vulnerable than ever to infectious diseases, and especially to ERADs such as AI, which typically cause catastrophic losses.

Effective control and eradication strategies must be based on: sanitation, isolation rearing; early detection; elimination of affected flock(s) without spread; premise decontamination and adequate surveillance.

To prevent and control infectious diseases, the ERAD Committee recommends the following steps be incorporated into a company's biosecurity guidelines at their discretion:

1. Anyone coming from a country or state having an ERAD (especially AI or VVND) should not come into contact with live poultry for a period of 7 days following the individual's arrival in Mississippi.
2. Wear freshly washed and disinfected boots or disposable boots when visiting a farm or when moving from one farm to another. Require visitors to do the same. Optimally, also change to fresh, clean coveralls, and headgear.
3. Equipment shared between farms should be cleaned, washed, and disinfected before it is used on another farm.
4. Thorough house cleaning, followed by vigorous washing, often is more important than the disinfectant you use. You can't disinfect dirt! Dirt or other filth takes the punch out of a disinfectant's chemical activity. Scrub brushes, pressure sprayers, orderliness (have a plan), and a lack of clutter are essential ingredients. Remember, disease-causing microbes are invisible, numerous, and hard to kill.
5. Plan (location of buildings) and engineer your operations to prevent situations that may expose your birds to disease.
6. Good ventilation pays! Fresh air dilutes the microbe population and reduces disease build-up.
7. Do business with operations and firms that consistently practice high biosecurity standards.
8. Practice all-in, all-out management if possible.
9. Promptly bury, compost, or burn dead birds.
10. Keep free-flying birds, waterfowl, stray poultry, pets, and varmints away from your commercial flocks. (Waterfowl are natural carriers of avian influenza and other poultry diseases.)
11. Employ aggressive, continuous control measures for rodents and insects, especially flies.
12. Don't mix various species of birds.
13. Minimize the mixing of different age birds.
14. Minimize the mixing of progeny from breeders.
15. Post signs to restrict and control visitors.
16. Don't keep or allow caretakers to keep pet birds at home.
17. Use clean, disinfected coops when transferring breeder birds.
18. Clean water troughs daily and disinfect lines on a routine basis.
19. Don't let unsold birds accumulate. Don't give them to a neighbor.

REMEMBER: biosecurity expenditures should be viewed not as unnecessary costs, but as short- and long-term investments in a safer, more profitable future.

POSSIBLE MEANS OF INTRODUCING AN ERAD INTO MISSISSIPPI

1. Trucks hauling any of the following to-or-from an adjacent area having an active ERAD or driving through such an area:

poultry	grain
feed	feed ingredients (including animal and poultry byproducts)
eggs	
limestone	livestock (poultry, cattle, hogs)
fertilizer	stone and gravel
manure	hay
offal	equipment
	supplies

2. Wild fowl hunters who have contact with poultry.
3. Dressing wildfowl on poultry farms.
4. Farm ponds (on poultry farms) that attract wildfowl.
5. Dogs and cats on farms that have contact with wildfowl.
6. Reused containers (egg cases, coolers, etc.) and pallets that are not cleaned and sterilized.
7. Vaccinating, debeaking, and catching crews from ERAD area.
8. Salespeople covering ERAD area as well as Mississippi.
9. Tours, vacations and visits by Mississippi poultry industry personnel to ERAD area and people from ERAD area coming to Mississippi and contacting poultry.
10. Artificial inseminators from ERAD area.
11. Craftsmen (millwrights, carpenters, electricians, plumbers, mechanics, etc.) who work both Mississippi and ERAD area.
12. Farm shows, pigeon races.
13. Fighting cock contests.
14. Utility meter readers, postal delivery from farm to farm.
15. Backyard flocks exposed to wild birds, visitors and highway traffic.
16. Poultry fanciers, pigeon clubs - who buy, sell and swap stock.
17. Poultry auctions in Mississippi and those in nearby states where people from Mississippi might buy and sell birds.
18. Movement of broilers, started pullets and spent fowl from-or-to Mississippi. Be especially concerned about sanitation of personnel, vehicles, coops or cages, and equipment used.
19. Purchase or trading pet birds – especially concerned about VVND.
20. Feed for table egg operations, dairy or livestock operations; supplies; and/ or farm equipment hauled between Mississippi and area with an ERAD.
21. Livestock (veal calves, hogs, goats, etc.) being moved to and from Mississippi and area with ERAD.
22. Deer, wild turkey, pheasant, etc. hunting trips into contaminated areas.
23. Farm and equipment sales (auctioneer type) in contaminated area that Mississippi growers attend or similar sales in Mississippi that out-of-the-area growers might attend.

ACTIVITIES OF POULTRY COMPANY. Each company should:

1. Provide educational programs to train all hands-on workers (from breeders through hatcheries, grow-out, and slaughter) in all levels of poultry and egg production on methods of disease transmission and biosecurity procedures.
2. Review ERAD manual and supply ERAD kit to flock supervisors, etc.
3. Publish and distribute biosecurity procedures for each industry worker, customized to apply to their particular type job.

REQUIREMENTS OF COMPANY QUARANTINE (UNOFFICIAL)

This should be done by a company when it suspects a disease threat within its operation.

1. Eliminate all service and other visits to that farm (including supervisor) – except under direct control of flock supervisor.
2. Fully inform grower of the problem and danger involved.
3. Grower and family restrictions:
    a. Limit flock management to specific individuals.

b. Fully inform (through discussion, printed material, and demonstrations) these individuals on procedures for clothing, disinfection, dead bird disposal, and limitations on their off-farm visiting. No other farms can be visited and grower should not come in contact with other growers.

c. Other family members working away from the farm must not enter poultry house.

d. Family members who work off the farm must not have contact with any other poultry or pet birds.

4. Post quarantine signs at entrance to farm and on poultry house doors.

5. Procedure for feed deliveries – assisted by flock supervisor:

   a. Make delivery a last stop for unloading split load.

   b. Driver must not enter poultry house.

   c. Driver must wear plastic boots.

   d. Keep truck doors closed during unloading operation to keep dust, flies and other insects out.

   e. Spray aerosol insect killer and disinfectant inside the truck cab.

   f. Truck must be run through truck wash before delivering feed to another farm.

6. Birds will be moved according to procedures outlined by ERAD Committee – including dead bird disposal.

7. ERAD Committee will outline procedures for cleaning and disinfection (C&D) of house(s) after removal of birds.

8. Withhold placements on that farm and adjacent farms until suspect is diagnosed, and a plan established for restocking.

MISSISSIPPI BOARD OF ANIMAL HEALTH (MBAH) ACTIVITIES

1. Continue requiring permits or licensing of dealers.

2. Monitoring for ERAD (to be implemented by Mississippi Board of Animal Health as conditions warrant)

   a. Collect blood samples from diseased birds submitted to diagnostic laboratories.

   b. Collect blood samples from broilers at time of processing.

   c. Collect blood samples from breeders.

   d. Samples will be tested for serological evidence of disease. As needed, positive samples will be submitted to USDA's National Veterinary Services Laboratory at Ames, Iowa, for confirmation.

WHAT TO DO IF AN ERAD IS SUSPECTED BY GROWER

1. Immediately telephone flock supervisor of suspicion.

2. Flock supervisor immediately gives this flock top priority and notifies company management.

ERAD KIT. All flock supervisors should have the following items **in their vehicle**:

copy of *THE MISSISSIPPI PLAN* (this manual)	clean coveralls
disposable masks	disposable headwear
rubber boots (or good quality disposable boots)	disposable gloves
disinfectant and insecticide spray	plastic garbage bags
bucket	brush

PROCEDURE FOR HANDLING SUSPECT FARM PREMISES BY FLOCK SUPERVISOR

1. Park vehicle well away from poultry house, preferably in a well-graveled or grassed area.

2. Put on all wearing apparel (clean), disinfect boots and gloves immediately on arrival. Recommended: coveralls, gloves, boots, hat, and mask.

3. If an ERAD is suspected by the supervisor, the grow-out manager or poultry health official within the company should be called.

4. Collect specimens for diagnosis using the following recommended procedures:

   a. Select fresh dead or kill live, symptomatic birds. Fluorescent antibody (FA) procedures require freshly killed (less than 2 hours) birds. Suspect birds (dead or alive) should be handled in such a manner as to prevent contamination of person, clothing, or vehicle with fecal matter or any other body discharges or feathers. **To prevent tearing of the bags, cut off beak and feet of dead birds at hock prior to putting birds in the plastic bag.**

   b. Tie bag closed.

   c. Disinfect outside of closed bag and place in second plastic bag.

   d. Disinfect outside of second bag.

   e. **Place boots, gloves, coveralls, mask, and hat in container of disinfectant** and transfer to plastic bag. **Repeat steps b, c, and d listed above.** Incinerate all disposable items on farm, if its available. Otherwise, place items in disposal pit on that farm.

f. Be careful to avoid contamination of vehicle.

g. **Do not move birds** until the appropriate diagnostic laboratory has been contacted for instructions on submitting specimens:

Mississippi Board of Animal Health
Veterinary Diagnostic Laboratory
Pearl, MS
800/ 852-1279 or 601/ 932-6771

h. When contacting laboratory, furnish laboratory personnel with map coordinates of the farm.

i. Launder coveralls.

j. Avoid contact with poultry or poultry industry personnel until there is complete decontamination of individual and car.

k. Run car through car wash and spray inside with disinfectant prior to visiting another farm.

l. If for any reason other assistance is needed, radio or telephone your company office.

m. Place company quarantine signs at driveway and on poultry house doors.

IF FLOCK SUPERVISOR UNSUSPECTEDLY ENCOUNTERS A POSSIBLE ERAD

1. Immediately call company office and seek assistance and instructions.
2. Serviceman to remain on farm until relieved and properly disinfected following procedures outlined above.

ACTION OF LABORATORY MAKING PRESUMPTIVE DIAGNOSIS OF ERAD

1. All diagnostic personnel should maintain ready alert and be prepared for emergency action – including names and phone numbers to call at night and on weekends.
2. Contact company involved regarding results – whether positive or negative.
3. Further action if POSITIVE laboratory presumptive diagnosis is made:
   a. If necessary, immediately contact and send appropriate sample(s) to NVSL in Ames, Iowa.
   b. Contact the State Veterinarian (888/646-8731).
   c. The State Veterinarian will notify all ERAD Committee members of positive diagnosis and its map coordinates.

THE MISSISSIPPI PLAN: YELLOW (LEVEL II)

PREVENTIVE PROGRAM TO AVOID THE INTRODUCTION OF AN ERAD IN EVENT OF DISCOVERY AND REPORTING IN A NEARBY AREA LIKELY TO POSE A THREAT TO MISSISSIPPI

1. MPA establish contact with appropriate agency or others to monitor the progress of the disease and its control.
2. Hold informational meetings with outside experts, government scientists, etc. early in outbreak.
3. Seek USDA assistance in critique of special preventive and control programs.
4. The companies establish truck washing stations for interstate traffic and list what vehicles need cleaning and sanitizing.
5. If threat warrants, implement appropriate farm visitation restrictions and other precautions.
6. Cease all direct contact with poultry-related people and businesses in contaminated area.
7. Distribute educational materials that have been previously developed in general context and currently modified to meet the specific features of an ERAD. Materials should be customized for practical impact on the variety of audiences targeted (e.g., growers, truckers, pet shops, repairmen, utility men, catching crews, producers, etc.).
8. Distribute list of potential loopholes and provide means for control.
9. Monitoring for ERAD
   a. Collect blood samples from diseased birds submitted to diagnostic laboratories.
   b. Collect blood samples from broilers at time of processing.
   c. Collect blood samples from breeders.
   d. Samples will be tested for serological evidence of disease. As needed, positive samples will be submitted to USDA's National Veterinary Services Laboratory at Ames, Iowa, for confirmation.
10. Conduct training courses for flock supervisors, hatchery personnel, etc.
11. Work with media to keep public and industry accurately informed of the danger of the threat and the need for good sanitation, isolation, and good management.

BIOSECURITY PROCEDURES for…

• Flock Supervisors

1. Always have ERAD kit available in your vehicle containing:

copy of *THE MISSISSIPPI PLAN* (ERAD manual)	clean coveralls
copy of this instruction sheet	disposable masks
boots (rubber or good quality disposable)	disposable headwear
plastic garbage bags	disposable gloves
disinfectant	insecticide spray
brush	bucket

2. Procedure for entering a poultry premise:
   a. Park vehicle well away from poultry house.
   b. Keep vehicle windows closed, spray inside vehicle with insecticide to kill flies.
   c. Put on clean overalls, headwear, mask, and boots.
   d. Disinfect boots.
   e. Disinfect equipment to be used – catching pen or hook, scales, etc.
3. Conduct intended business.
4. Consult *THE MISSISSIPPI PLAN* (ERAD manual) for specific instructions for removing sick or dead birds from premises (Green Level I, *Procedure for Handling Suspect Farm Premises by Flock Supervisor*).
5. Procedure for leaving the poultry premise:
   a. Leave all articles on farm that cannot be containerized, cleaned, and disinfected.
   b. Clean and disinfect equipment used.
   c. Remove boots.
   d. Remove coveralls and other disposable or washable items and place in plastic bags for disposal or laundering.
   e. Clean and disinfect boots.
   f. Wash hands, fingernails, and arms in disinfectant.
   g. Place all bagged and disinfected items in proper place in vehicle.
   h. Take vehicle to wash station and clean.
   i. Bathe and wash hair when arriving home.
6. It is the flock supervisors' responsibility to give clearance for authorized visitors to the premises and to assure they are properly attired in protective clothing and boots.

BIOSECURITY PROCEDURES for…

• Flock Growers

The ERAD Committee strongly recommends the following for poultry growers (including game birds, waterfowl, exhibition flocks, sporting birds and small farm flocks):

1. Keep poultry houses locked; fasten from inside while inside.
2. Resident flock manager should have clothing (including shoes, boots, headwear and gloves) used when caring for flocks separate from those worn off the farm.
3. Flock manager and other caretakers should not visit any other poultry flocks.
4. Do not allow visitors in or near the poultry houses.
5. After caring for the flock, change clothes completely and wash hands and arms before leaving premises.
6. Essential visitors such as owners, fuel and feed delivery drivers, meter readers, poultry catchers and haulers, and service personnel must put on protective outer clothing including boots and headwear prior to being allowed near the flocks.
7. Monitor vehicles entering premises for poultry pickup or delivery, feed delivery, fuel delivery, etc. to determine if they have been scrubbed down and disinfected prior to entering. Pay particular attention to the undercarriage and tires.
8. All coops, crates and other poultry containers or equipment must be cleaned and disinfected prior to use and following use.
9. Sick or dying birds should be submitted to one of the laboratories mentioned in Green Level I, page 7 of *THE MISSISSIPPI PLAN* (ERAD manual) for a diagnosis. Commercial growers should contact their flock supervisor.
10. Dead birds must be properly disposed of by burial, composting, or incineration on the premises.
11. Persons handling wild game (especially waterfowl) must change clothes completely and bathe prior to entering poultry premises.
12. Keep "restricted" signs posted at drive entrances. These are available from flock supervisors.

BIOSECURITY PROCEDURES for Operators of…

- **Feed Delivery and Pickup Trucks**
- **Chick Delivery Buses**
- **Egg Pickup Vehicles**
- **Feed Ingredient Delivery (including Grain)**
- **Miscellaneous Delivery Equipment**

CAUTION: If an ERAD occurs, each farm premise and each vehicle should be considered to be hazardous.

1. General Procedures
   a. Each driver will be furnished with clean coveralls, rubber boots, disinfectant, bucket and brush, spray insecticide, plastic bag for dirty coveralls, disposable or washable headgear, and paper towels.
   b. Driver and vehicle will visit only one farm per delivery or pickup. (Note: procedure is applicable for any highly pathogenic agents. If milder strains are present, procedure may be modified to permit multiple visits to those farms identified as such.)
      (1) Vehicle will carry only enough feed, ingredients, poults, chicks, or egg boxes for one delivery per trip.
      (2) Upon completion of delivery or pickup, vehicle will return immediately to terminal base for complete cleaning and disinfection.
2. Procedures For Farm Visit
   a. Drivers will operate only sanitized vehicle and will wear clean boots, washable or disposable headgear, coveralls or rain suits. This preventive equipment will be put on at the plant prior to delivery or pickup. Care must be taken to take only equipment that can be cleaned and sanitized after use.
   b. At no time, will feed or egg pickup drivers be allowed to enter a poultry house. Chick or poult delivery drivers who must enter a poultry house will be required to shower and wear clean clothing prior to making a delivery.
   c. Consideration should be given to how a driver will approach and enter each farm to minimize the chance of dust or manure contamination. When at all possible, avoid driving vehicle by the fan-side or downwind side of a poultry house; avoid driving through or near manure piles.
   d. Flies and other insects should be prevented from entering the vehicle; in the event they do, they should be killed with a spray insecticide prior to leaving the farm premise.
3. Procedures For Return From Farm Visit
   a. After every delivery or pickup, driver and vehicle must return directly to its operation base for complete cleaning and disinfection and disposal or disinfection of equipment. No intermediate stops between the farms and the operation base should be made for any reason.
   b. Upon the driver's return to the operation base, the driver will disinfect all rubber equipment, place washable clothing into a plastic sack and seal same, and place any disposable items that have been used in a plastic bag and seal for safe disposal. The vehicle used will be completely cleaned and disinfected inside and out before visiting another farm.
   c. Egg pickup drivers will not enter hatcheries or egg warehouses but can help unload from the truck. After unloading is completed, the driver will disinfect himself and his vehicle in accordance with step 3-b (*above*). All egg containers unloaded from the vehicle must be either completely disinfected or destroyed after eggs are processed. No traffic should occur between the storage area and the chick processing area without an effective disinfection procedure.

BIOSECURITY PROCEDURES for…

- **Catching Crews**

1. Precautions regarding clothing, headwear, and footwear.
   a. Dress in freshly laundered clothing.
   b. Wear clean, disinfected rubber boots or freshly laundered sneakers.
   c. Wear a new, disposable hairnet
2. Carry lunches in disposable bags and leave on premises. (Personnel not permitted to leave farm to go to local market for drinks, cigarettes, etc.).
3. Leave vehicle windows closed and spray with insecticide to kill flies.
4. After chickens have been caught and loaded:
   a. Load equipment and personnel into transport vehicle and return to wash station.
   b. Clean and disinfect catching devices, hooks, nets, fences and coops.
   c. Clean and disinfect inside and outside of vehicle used to haul crew and equipment.
   d. Crew members should remove clothing and shoes or boots, bathe, wash hair and dress in street clothes and shoes.
5. Do not enter other poultry premises unless you have changed into freshly laundered clothes and proper footwear.
6. Crew members must not visit flocks other than ones where you are working - unless steps 1-a and 1-b (above) are repeated.

BIOSECURITY PROCEDURES for…

• Live-haul Trucks, Trailers, Crews, Forklifts, Coops and Cages

1. Crews and equipment should visit only the farm from which flock is to be moved and then return to the base of operation for thorough cleaning and disinfection.
   a. Crew members should wear freshly laundered clothing.
   b. Wear clean, disinfected boots or freshly laundered sneakers.
   c. Carry lunches in disposable bags (personnel not permitted to leave farm to go to local market for drinks, cigarettes, etc.).
   d. Close vehicle's windows and doors and spray inside vehicle with insecticide to kill flies.
2. After chickens have been caught and loaded
   a. Live haul trucks and crew will go directly to processing plant.
   b. Remaining crew members will load equipment and return to wash station.
   c. Clean and disinfect all equipment used with this flock.
   d. Clean and disinfect inside and outside of all vehicles used.
   e. Clean and disinfect live haul tractor inside and out (paying particular attention to the undercarriage and tires), also the trailer after it has been unloaded and all coops, cages, chains, tarps and ropes.
   f. Dispose of all disposable (lunch bags, masks, caps, etc.) in containers provided for hazardous materials.
   g. Crew members should remove clothing and shoes or boots, bathe, wash hair and dress in street clothes and shoes.
3. Do not enter other poultry premises unless you have changed into freshly laundered clothes and proper footwear.
4. Crew members should not visit poultry flocks other than the ones where you are working.

BIOSECURITY PROCEDURES for…

• Pullet / Cockerel Movers
• Crews Moving Hens to Slaughter
• Vaccination Crews
• Debeaking Crews

1. Crews and equipment should be permitted only on the farm where flock is to be handled and then return to base of operation for thorough cleaning and disinfection. Only clean crews and equipment are permitted onto these farms.
   a. Crew members should wear freshly laundered clothing.
   b. Wear clean, disinfected boots or freshly laundered sneakers.
   c. Carry lunches in disposable bags (personnel not permitted to leave farm to go to local market for drinks, cigarettes, etc.).
   d. Leave vehicle windows and doors closed and spray with insecticide to kill flies.
2. After chickens have been caught or processed
   a. Live haul trucks and crew will go directly to farms where chickens are to be housed.
   b. Pullet and cockerel moving trucks and crews will go directly to farms where chickens are to be housed.
   c. Debeaking or vaccinating crew members will load equipment and return to wash station at base of operation.
   d. Clean and disinfect all equipment used with this flock.
   e. Clean and disinfect inside and outside of all vehicles used.
   f. Clean and disinfect live haul tractor, pullet/cockerel mover tractor, trailers after they have been unloaded and all coops, cages, chains, tarps and ropes.
   g. Clean and disinfect forklifts and other equipment used.
   h. Dispose of all disposable (lunch bags, masks, caps, etc.) in containers provided for hazardous materials.
   i. Crew members should remove clothing and shoes or boots, bathe, wash hair and dress in street clothes and shoes.
3. Do not enter other poultry houses unless you have changed into freshly laundered clothes and proper footwear.
4. Crew members should not visit poultry flocks other than the ones where you are working.

BIOSECURITY PROCEDURES for…

- **Equipment Service Personnel**
- **Electric and Gas Representatives**
- **Carpenters and Plumbers**
- **Exterminators**
- **Others Working Inside Poultry Houses**

1. No entry is permitted in or near poultry houses without prior authorization from the contracting poultry company and a biosecurity clearance on the scene by the flock supervisor.
2. Park service vehicle well away from poultry house; keep windows closed.
3. Required clothing and footwear,
   a. Freshly laundered or disposable coveralls.
   b. Rubber boots, cleaned and disinfected.
   c. Cap and mask (disposable).
4. Lay out only equipment and tools required for this job,
   a. With wash cloth dipped in disinfectant and wrung damp-dry, wipe all surfaces of equipment and tools to be taken in poultry house.
   b. Any additional tools discovered to be needed later have to go through the same procedure.
5. When work is completed,
   a. Clean and disinfect all tools and equipment removed from poultry house.
   b. Remove boots, clean and disinfect.
   c. Remove and dispose of disposable items in plastic bag provided.
   d. Remove coveralls and place in plastic bag provided.
   e. Wash hands and arms in disinfectant solution.

THE MISSISSIPPI PLAN: RED (LEVEL III)

PLAN OF ACTION IF AN ERAD IS CONFIRMED IN MISSISSIPPI

1. If the ERAD is AI, follow the USDA AI Technical Advisory Committee's Recommendations.
2. The following items must be resolved as soon as possible by Mississippi Board of Animal Health in consultation with the MPA ERAD Committee, depending on the disease and the potential economic threat that it represents:
   a. Establish quarantine boundaries.
   b. Establish sites and methods for disposal of flocks and litter from ERAD breaks.
   c. Dispose of flock and litter.
   d. Thoroughly clean and disinfect premises.
   e. Centralize all industry disease reporting and information releases through College of Veterinary Medicine. State and federal authorities will disseminate information regarding regulatory activities.
   f. Active vehicle washing and decontamination stations.
   g. Establish smallest convenient operating unit.
   h. List details on limiting traffic, handling trucks and ingredient hauling.
   i. Monitoring for ERAD (especially AI).
      1) Test all flocks as blood samples are routinely submitted to diagnostic laboratories.
      2) All commercial poultry (including table egg flocks) must test negative within 7 days PRIOR to movement.
      3) Require testing of all exhibition flocks in conjunction with other required testing (shows, sales, etc.) through Mississippi Board of Animal Health personnel.
      4) Require testing of all backyard flocks and confined wild birds through Mississippi Board of Animal Health personnel. (Develop mailing list of all backyard flocks.)
      5) Submit specimens for laboratory diagnosis on all suspect disease outbreaks.
      6) Actively pursue poultry inventory data by updating map and necessary text material of growers in Mississippi.
   j. Determine when emergency is concluded so that normal business can be resumed.

CLEANING AND DISINFECTION (C & D) PROCEDURES FOR POULTRY PREMISES CONTAMINATED WITH AN ERAD

Until the flock has been properly depopulated and the house and all equipment have been totally and completely cleaned and disinfected, any disease present can be easily spread to other poultry. During the cleaning process, contact with the house or any of its contents until disinfection is complete, can be a source of disease spread.

The following steps are recommended as guidelines to achieve containment and destruction of ERADs:

1. Following removal of a flock for approved disposition, the poultry houses shall be closed and nothing removed including feed, litter, equipment, etc.
2. Spray premises inside and out with an adult fly knockdown product.
3. Apply products for effective rodent control using baits and a fumigant for burrows.
4. Treat interior of house and litter with a broad-spectrum insecticide to control beetles, etc.
5. Remove all litter, manure, and feathers for disposal in a safe manner, preferably to an on-farm site by:
   a. burial
   b. composting under heavy plastic, well away from houses
   c. spread on fields, well away from houses and neighbors and immediately plowed under
6. Completely wash down the ceiling, walls, curtains, windows, fans, equipment, floors with a sanitizing detergent in ample water. Rinse with water under pressure until "like-new" clean. Repeat the wash and rinse steps where necessary.
7. After inspected and certified as adequately clean, thoroughly wet all surfaces of the equipment and houses with an approved disinfectant. Spray the floors and lower wood and block structures with a phenolic or cresylic product until wet. Use a disinfectant that is USDA approved for the specific disease.
8. Thoroughly clean and disinfect work room, egg room, pump room and all storage areas.
9. Rid outside area of all spilled litter, manure, etc.; spray disinfectant outside of curtains, windows, sills and fan shutters; and disinfect all doorways, walks and drives out 15 feet from house with phenolic or cresylic disinfectant.
10. Spray disinfectant on areas outside of house that are dust coated by exhaust fans.
11. Thoroughly clean and spray disinfectant all loaders, trucks, trailers, spreaders, tractors, hand tools, etc. used in this C & D process.
12. Conduct monitoring tests and wait 30 days before repopulating.

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