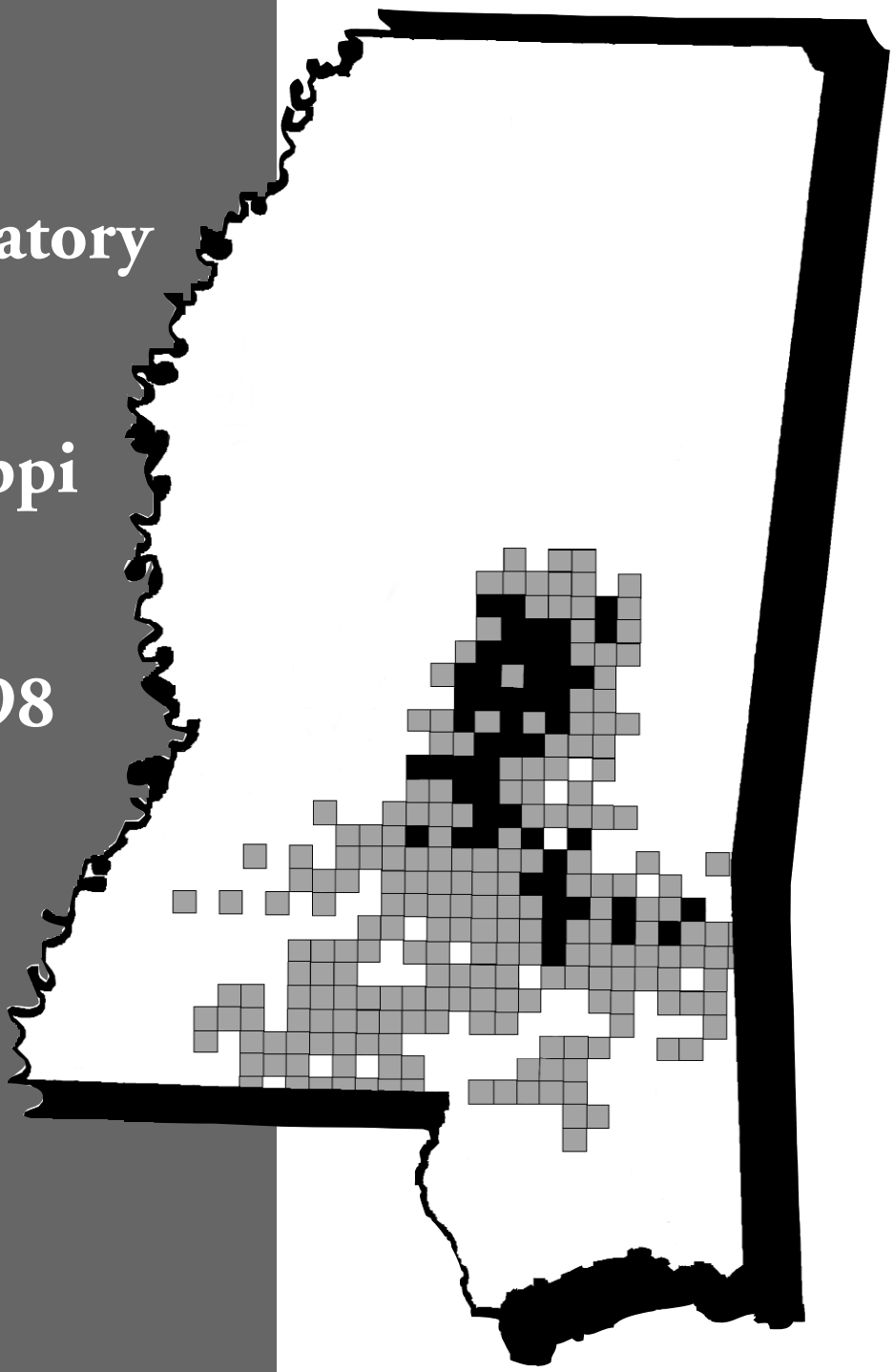
Bulletin 1127 February 2003

The Respiratory Outbreak in Mississippi Broilers During 1998



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On the Cover: Map of Mississippi showing the grid system used to locate the major broiler production area in the state (grey). Also shown (in black) are the areas that were involved in the 1998 respiratory outbreak.

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This study was supported by the MSU College of Veterinary Medicine and the Mississippi Agricultural and Forestry Experiment Station. For more information, contact Dr. Montgomery by telephone at (662) 325-1279 or by e-mail at montgomery@cvm.msstate.edu. Bulletin 1127 was published by the Office of Agricultural Communications, a unit of the Division of Agriculture, Forestry, and Veterinary Medicine at Mississippi State University.

The Respiratory Outbreak in Mississippi Broilers During 1998

Summary

Two hundred and fifty-eight cases were received from a respiratory outbreak that involved the major broiler-growing region of Mississippi during 1998. Tissues collected from these cases were assayed for viruses using a combination of embryo passage, indirect fluorescent antibody (FA), and polymerase chain reaction (PCR) techniques. Additional data collected from these cases included date received, identity of growout company involved, age of birds, strain(s) of infectious bronchitis virus (IBV) in vaccination program, infectious bursal disease (IBD) vaccination status, condition of samples received, any respiratory lesions noted, and the geographic location of farm. These data were analyzed to determine if any epidemiological associations occurred. Based on the numbers of viruses detected and especially on the number of viruses detected from flocks not vaccinated with the same strain, Arkansas and, to some extent, Connecticut IBV, were the principal agents in this outbreak. *Ornithobacterium rhinotracheale* and *Bordetella avium* were isolated from a few cases; however, their presence did not seem to exacerbate those cases. In general, the number and distribution of (1) cases received, (2) cases positive for virus, and (3) viruses detected were proportional to the epidemiological factors monitored.

Introduction

Early in January 1998, broiler company personnel noticed an increase in respiratory noise among broilers grown in Mississippi. Samples, primarily tracheas, were submitted to the diagnostic laboratory to identify what infectious agent(s) might be involved. A combination of embryo passage, indirect fluorescent antibody (FA), and polymerase chain reaction (PCR) were used to isolate and identify any viruses detected. In addition, procedures were conducted to isolate any *Mycoplasma gallisepticum*, *M. synoviae*, *Ornithobacterium rhinotracheale*, or *Bordetella avium* present.

Over the course of the year, 258 cases of respiratory disease in broilers were received. This report evaluates the procedures used to render the diagnoses and documents other specifics of the outbreak, including its epidemiological and spatial aspects.

Materials and Methods

Case Submissions

For purposes of this report, a case is defined as any laboratory submission/accession from a flock of commercial poultry having signs of respiratory disease (snicking, coughing, etc.) and from which respiratory tissues (trachea with or without lung) were received.

Most cases received were routed through Central Laboratories, Inc. (Forest, Mississippi). Approximately half of the cases were submitted as whole birds and necropsied. Any respiratory lesions noted at that time were scored on degree of severity, as follows: 0 = none, 1 = tracheal inflammation, 2 = airsacculitis (with or without tracheal changes), or 3 = pericarditis and/or perihepatitis (with or without tracheal changes and or airsacculitis). For the remaining cases, tracheas (and, on occasion, lungs) were collected on the farm and submitted by growout company personnel.

Tracheas from all cases were sent to the College of Veterinary Medicine for pathogen detection. If sent on the day of or the day following collection, tracheas were shipped chilled (5°C); otherwise, they were frozen (-20°C) and shipped chilled later. Upon receipt, tracheas were opened longitudinally and their gross condition scored on a case basis. A score of 0 was assigned if no appreciable changes were noted. A score of 1 was added for each of the following: moderate amount of blood, moderate amount of mucous/exudate, any desicca-

tion/dehydration, any contamination (with feathers, feces, feed, etc.), or any necrosis noted. Thus, tracheal condition scores ranged from 0 to 5.

Any excess exudate and/or debris present were removed from the tracheas. Next, the tracheal mucosa was scraped and the contents placed in 5 ml of tryptose phosphate broth (TPB). After vortexing, the tube was sampled for *Ornithobacterium rhinotracheale*, *Mycoplasma gallisepticum* (MG), and *M. synoviae* (MS) and then frozen (-65°C). After a total of 3 freeze/thaw cycles, the material was passed through a 0.22-µm syringe filter and a 1:10 dilution of the filtrate made in TPB containing 100 IU/ml of penicillin G and 100 mg/ml of streptomycin. Once mixed, the antibiotic-treated sample was allowed to stand at 22°C for 45 minutes.

Embryo Inoculation for Virus Detection

Five 10- or 11-day-old embryonated eggs from a commercial specific-pathogen free (SPF) source (Hy-Vac, Inc., Gowrie, Iowa) were inoculated via the chorioallantoic sac (CAS) at the rate of 0.2 ml of antibiotic-treated sample per egg. The eggs were sealed, incubated at 37°C, and candled daily. After 2 days of incubation, 2 eggs were removed and their chorioallantoic membranes (CAM) and CAS fluids harvested for FA testing and PCR amplification, respectively. After 6 days of incubation, CAS fluid was sampled from all remaining embryos and pooled. Embryos dying during the 6-day period were 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 phosphate-buffered saline (PBS) to determine the presence of hemagglutination (HA) agents. Hemagglutination-negative eggs were opened and their embryos examined for lesions.

If negative for HA activity and embryo lesions, harvested CAS fluids were frozen (-65°C) and thawed 3 times and then inoculated for a subsequent passage. CAS fluid from embryos that died in the previous passage and were free of bacterial contamination were added to the pool of CAS fluids used as the inoculum. In the absence of any changes or suggestive embryo lesions, each submission was passaged in embryos a total of 4 times before being considered negative.

Any HA-positive CAS fluid was tested for the presence of Newcastle disease virus (NDV) and avian influenza virus (AIV) using an agar gel precipitin (AGP) test. Embryos that had lesions suggestive of IBV, such as dwarfing, stunted down, and/or reflexively bent toes were also tested for the presence of adenovirus (Group I) using the AGP test. IBV suspects were passaged 1 additional time in embryonated eggs and 2 embryos from this passage were harvested after 2 days of incubation and tested by FA and PCR for IBV confirmation.

Bacterial Isolation

A loopful of the original sample tube containing the TPB-tracheal suspension 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 visually for small colorless colonies typical of *O. rhinotracheale (1)*. Any colonies that were Gram-negative pleomorphic rods were screened for cytochrome C oxidase and catalase activity, motility, MacConkey agar growth, indole production, and the ability to reduce nitrate. Isolates that were oxidase-positive and negative for catalase, motility, MacConkey agar growth, indole production, and nitrate reduction were further identified by selected biochemical tests and compared with published results *(4,13)*.

All samples were 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 was used to inoculate both MG and MS broth at a ratio of 1:10 *(2)*. Inoculated tubes were incubated at 37°C for 21 days. During that period, the tubes were inspected daily and compared with uninoculated control tubes for color change in the phenol red indicator. Any tube turning color was subcultured onto MS agar. These plates were incubated for 7 to 14 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.

Agar Gel Precipitin Testing

Harvested CAS having HA activity was tested against virus-specific antiserum to Newcastle disease virus (SPAFAS, Inc., Norwich, Connecticut) and avian influenza virus (USDA, Ames, Iowa) in an agar-gel precipitin (AGP) test. CAS harvested from embryos with lesions suggestive of IBV was also tested against adenovirus antiserum (SPAFAS, Inc.). Once loaded, AGP plates were placed in a humidified container, incubated at 22°C, and inspected visually for precipitin lines after 24 and 48 hours of incubation.

Fluorescent Antibody (FA) Testing

With minor modifications, the indirect FA test, using murine monoclonal antibodies (MAbs) obtained from Dr. S.A. Naqi of the College of Veterinary Medicine at Cornell University, was performed as previously described *(7,14)*. Briefly, harvested CAM were frozen in embedding medium (O.C.T. Compound, Tissue-Tek®, Sakura, USA) and sectioned at 10 µm using a cryostat. The sectioned membranes were placed on a glass slide and fixed for 5 minutes in cold acetone. Fifty-microliter volumes of group-specific (S2 and M antigens) or serotype-specific MAbs against Massachusetts 41, Arkansas 99, or Connecticut antigens were individually reacted with the fixed sections for 30 minutes at 37°C. The MAbs were washed 3 times from the sections with PBS (0.01M, pH 7.2) for 5 minutes each wash. After washing, bound MAbs were detected using 50 µl of fluorescein-labeled goat antimouse heavy and light chain specific IgG (Southern Biotechnology Associates, Inc., Birmingham, Alabama) for 30 minutes. All antibodies were used at a 1:50 dilution. After washing with PBS, the slides were mounted in PBS containing 15% glycerol and examined for specific fluorescence using an epifluorescence microscope (Laborlux 12, Leitz, Wetzlar, Germany).

Polymerase Chain Reaction (PCR) Testing

Ribonucleic acid (RNA) extraction was performed as previously described *(3)* 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 complimentary deoxyribonucleic acid (cDNA) was amplified using serotype-specific primers described by Keeler et al. *(6)*. The PCR products were separated by electrophoresis in 1.5% agarose with 0.01% GelStar (FMC BioProducts, Rockland, Maine) *(6)*.

Epidemiological and Spatial Data

The following information was requested at time of sample submission: company complex/division name, grower or farm name, and age of birds at time of submission. Periodically, broiler company representatives were queried for details of their NDV, IBV, and infectious bursal disease (IBD) vaccination programs, including the age of birds when vaccinated and strain of vaccine(s) used. In addition, company representatives were provided a map of Mississippi overlaid with an X-by-Y grid system, each grid measuring 6 X 6 miles or 36 square miles. The companies were asked to supply the grid coordinates of the farm of origin for all cases. In addition, they were asked to give the total number of farms in each of the grids they occupied.

Analyses of Data

The total number of respiratory cases received during the year was used to compare FA and PCR methodologies for diagnosing and typing IBV. Using subsets of the total cases received, the data were also scrutinized to look for associations between viruses detected and the epidemiological attributes requested: month received, contracting company, age of birds (in weeks), strains of IBV in vaccination program, whether or not the birds had been vaccinated with IBD virus, tracheal scores, and necropsy scores. For this analysis, the total number of cases received was reduced by (1) those submissions of SPF-source Leghorn chickens that had been placed on broiler farms as sentinel birds and (2) by those submissions in which broilers were sampled from the same farm twice during the same growout period (flock). The reason for the latter submissions being omitted was to reduce those submissions that might not be independent of each other. When the latter did occur, the second of the 2 submissions was omitted unless either the first submission was virus-negative and the second was positive, or the second submission had a greater array of viruses detected.

The grid-system coordinates of the submitting farms were analyzed by a computerized geographic information systems (GIS) program (ArcView GIS®, ESRI, Inc., Redlands, California) for spatial distribution of the viruses detected. A density distribution of broiler farms by their placement capacity and the number of cases positive for Arkansas and Connecticut IBV diagnoses was calculated using the Spatial Analysis® function of that program.

RESULTS AND DETECTION

Cases Received and Viruses Diagnosed

Altogether, 258 cases were received during 1998. The cases originated from flocks under contract to 14 of the 15 poultry complexes — representing all 8 of the commercial companies — raising broilers in the state. One hundred and fifty-two (59%) of these cases were positive for 1 or more viruses, yielding a total of 258 viruses (Table 1). Two viruses in particular, the Arkansas and Connecticut strains of IBV, accounted for a large percentage of the positive cases. Arkansas IBV was detected in 118 (46%) of all cases submitted and was clearly the most prevalent virus detected. This was followed by the Connecticut strain, which was detected in 82 (32%) of all cases submitted. The remaining viruses detected included NDV, in 24 cases (9%); Massachusetts IBV, in 21 cases (8%); and adenovirus, in 13 cases (5%).

The practice of using modified-live virus strains in vaccination programs can confuse the definitive diagnosis when homologous strains are considered the cause(s) of the problem. This, undoubtedly, could have been a factor here; however, short of testing the nucleic acid sequence of recovered IBV, it is impossible to determine whether the isolates were of vaccine or field origin. The NDV viruses recovered, while not typed, were assumed to be lentogenic (mild) strains of the virus as no untoward syndromes suggestive of mesogenic or velogenic (more virulent) strains were reported from the field.

Regarding the adenovirus detected here, group I adenoviruses have frequently been isolated over the years by this laboratory during the course of detecting respiratory viruses in field samples. Prevailing opinion is that these viruses are not considered to be primary respiratory pathogens *(8)*.

Table 1. Cases received and viruses detected during the 1998 respiratory outbreak in Mississippi broilers.[1]

Cases [2]	Total [3]	
Cases received	258	
Cases containing 1 or more viruses	152	(59%)
Cases containing:		
IBV - Arkansas	118	(46%)
IBV - Connecticut	82	(32%)
IBV - Massachusetts	21	(8%)
NDV	24	(9%)
Adenovirus	13	(5%)
TOTAL viruses detected	258	

[1]Viruses detected by a combination of embryo passage, fluorescent antibody, and polymerase chain reaction.
[2]Abbreviations: IBV = infectious bronchitis virus, NDV = Newcastle disease virus.
[3]Percentages based on 258 cases received.

Protocol Used to Detect IBV

The combination of all 3 techniques (embryo passage, FA, and PCR) gave the highest number of IBV detected. One hundred and thirty-three of the 258 cases (52%) were virus-positive when this combination was used, compared with 114 (44%) by embryo passage alone and 105 (41%) by FA/PCR without higher embryo passages. In general, there was good agreement between embryo and FA/PCR protocols. Two hundred and eleven (81%) of the 258 cases were either virus-negative by both protocols (125 cases, 48%) or virus-positive by both (86 cases, 33%). However, relying only on FA or PCR analysis of the first 48-hour CAS or CAM samplings without additional blind embryo passages for those that were FA/PCR negative would have resulted in 28 of the 258 cases (11%) being incorrectly diagnosed as negative. Likewise, 19 cases (7%) would have been incorrectly diagnosed if embryo passages had been the only technique of detection used. Eleven of these 19 cases were negative because the presence of NDV activity in the embryos precluded any additional passages to detect IBV.

Table 2 compares the results of FA and PCR for the detection and typing of IBV conducted on the 48-hour postinoculation (PI) materials from the first passage of field material in embryonated eggs. Overall, these results indicate that PCR detected more viruses than FA, which was primarily due to the former detecting more multiple-virus combinations. In addition, PCR detected IBV in 32 cases that were negative by FA, while the reverse situation (i.e., FA positive and PCR negative) occurred in only 3 cases. One other difference was that while all cases that were IBV group-positive by PCR were further typed to 1 or more of the 3 IBV serotypes, there were 3 cases that were IBV group-positive by FA for which a serotype could not be identified.

Based purely on the number of IBV recovered, it would appear that a combination of all 3 identification techniques afforded the best results. Our results indicate that the optimum protocol would be to inoculate embryonated eggs with suspect material, harvest a few of the embryos at 48 hours PI and analyze for the presence of IBV via FA and/or PCR. The remaining embryos should be incubated to complete a 6- to 7-day passage and harvested for up to 3 additional embryo passages in the event the 48-hour samplings proved to be negative by PCR and/or FA. If any of the higher passages produce lesions typical of IBV, an additional 48-hour passage could be made and PCR and/or FA used to confirm the viruses' identities.

It should be noted that all 3 of these techniques — embryo passage, FA, and PCR — have features that complicate a diagnosis. Viruses such as NDV and IBV, which are available as modified-live virus vaccines, are generally produced in embryonated eggs. Theoretically, embryo passaging favors the recovery of vaccine strains, whereas less adapted strains, which could have been present and the real cause of the problem, are less readily detected. FA and/or PCR are based on detecting antigen (FA) or specific nucleic acid sequences (PCR) rather than complete virions. Consequently, it is possible that these 2 techniques may detect residual fragments of a nonclinical exposure or a vaccine strain of the virus rather than detecting the virus that precipitated the clinical disease.

Table 2. A comparison of indirect fluorescent antibody (FA) and polymerase chain reaction (PCR) to detect and type infectious bronchitis virus (IBV).[1]

FA results[2,3]		PCR results[3,4,5]									
		Negative	IBV group								Total
			(only)	+ A	+ AC	+ C	+ M	+ MA	+ MC	+ MAC	
Negative		**153**	0	15	12	4	1	0	0	0	185
IBVgroup	(only)	0	**0**	1	2	0	0	0	0	0	3
	+ A	1	0	**21**	25	0	0	0	0	2	49
	+ AC	0	0	0	**1**	0	0	0	0	0	1
	+ C	2	0	2	2	**3**	0	0	0	1	10
	+ M	0	0	0	1	0	**0**	1	2	5	9
	+ MA	0	0	0	0	0	0	**0**	0	0	0
	+ MC	0	0	0	0	0	0	0	**1**	0	1
	+ MAC	0	0	0	0	0	0	0	0	**0**	0
Total		156	0	39	43	7	1	1	3	8	**258**

[1]Tests were conducted using field samples passaged for 48 hours in embryonated chicken eggs.
[2]Chorioallantoic membrane (CAM) used for FA analysis.
[3]Code: A = Arkansas, C = Connecticut, M = Massachusetts (strains of IBV).
[4]Numbers in bold type indicate agreement between tests.
[5]Chorioallantoic sac (CAS) fluid used for PCR analysis.

Bacterial Isolations

Six cases were positive for *O. rhinotracheale*. Although each originated from different farms, all the farms were under contract to the same broiler company. All 6 cases were received over a 10-week period starting the end of September. Various viruses were also detected in 4 of the 6 *O. rhinotracheale*-positive cases. On the other hand, 4 cases were positive for *Bordetella avium*; all 4 were received over an 8-week period, starting in October. None of the *B. avium*-positive cases were also *O. rhinotracheale*-positive; however, 2 of the *B. avium* isolations were from the same company having the 6 *O. rhinotracheale*-positive cases. Two of the 4 *B. avium*-positive cases also had viruses detected in them. Conversations with representatives of those companies from which these 2 bacteria were recovered failed to disclose any additional or unique clinical manifestations over and above those experienced by other farms in the outbreak.

The status of both *B. avium* (known in the 1980s as *Alcaligenes faecalis*) and *O. rhinotracheale* as respiratory pathogens in domestic chickens is uncertain. *B. avium* has been recovered over the years from poultry; however, while this organism is considered a primary respiratory pathogen in turkeys *(11)*, attempts to establish a similar role for it in chickens have been less successful *(5,9)*. *O. rhinotracheale* is a fairly recent diagnostic entity in poultry circles. While some claim the organism to be a respiratory pathogen *(12)*, others have questioned this claim *(10)*.

No MG or MS were isolated from any of the cases submitted.

Epidemiological Analyses

Fifty of the 258 total cases were excluded from these analyses for various reasons, including 32 cases in which SPF Leghorns served as sentinel birds, 17 cases that were duplicate submissions from the same flock, and 1 case in which egg passage lesions suggested the presence of an IBV-like agent that was unidentifiable by either FA or PCR. This left a maximum of 208 unique cases with all or some of the epidemiological parameters requested.

Although the analyses that follow cover all 5 of the viruses detected — Arkansas, Connecticut, and Massachusetts strains of IBV, NDV, and adenovirus — the focus will be on the first 2 of these viruses, because they represent the greatest number of those detected. Throughout this discussion, it should be noted that, for most factors, both numbers of the cases positive for virus and numbers of viruses detected were, with few exceptions, proportional to the total number of cases submitted.

Month received (data available: 208 cases submitted, of which 124 cases were positive for virus and, from these, a total of 204 viruses were detected; Figure 1A). Overall, positive case submissions were more numerous in cooler months with a definite respite during the summer. In fact, only 5 cases were received in June, none in July, and although 17 cases (8.2% of all cases received) were received in August, only 5 were positive for virus. Four months — February, May, November, and December — accounted for 57% of the cases submitted, 65% of virus-positive cases, and 67% of the viruses detected. These same months accounted also for more than 66% the numbers of each of the Arkansas (69%) and Massachusetts (77%) IBV strains detected.

Broiler company (data available: 208 cases submitted, 124 virus-positive cases, 204 viruses detected; Figure 1B). Two companies (coded S and W) submitted a total of 100 cases, which was nearly half (48%) of all cases submitted. Two other companies (T and U) submitted 54 (26%) of the cases received, while the remaining 26% of cases originated from the 4 remaining companies (V, X, Y, and Z).

Despite the variation in numbers of cases submitted by each of the companies, the percentage of positive to total number of cases was fairly uniform for 7 of the 8 companies (50% to 67%). Company Y, with the fewest number of submissions (total = 11), had the highest percentage of cases positive (82%). Also, despite the variation in cases submitted, the proportion of cases positive for Arkansas and Connecticut IBV relative to the total viruses detected for the 8 companies were generally uniform, ranging from 41% to 50% for Arkansas and from 25% to 37% for the Connecticut strain.

The first 2 cases that were positive for Arkansas with or without Connecticut occurred during January. Both cases originated from the same company, 1 of the only 2 using Arkansas IBV in its broiler vaccination program at that time. During February, flocks submitted from 5 additional companies were positive for Arkansas IBV alone or together with Connecticut IBV. Only 1 of these companies was using Arkansas IBV in its vaccination program at that time. The remaining 2 companies had their first Arkansas IBV alone or together with Connecticut IBV positives diagnosed in March and May, respectively. Neither of these companies included Arkansas IBV in their vaccination programs at that time.

Age of broilers (data available: 205 cases submitted, 124 virus-positive cases, 201 viruses detected; Figure 1C). Ten (5%) of these cases were received from birds that were 1 week or 8 weeks of age, while another 39 (19%) cases were from broilers that were 2 or 3 weeks of age. The bulk (76%) of these cases originated from birds that were between 4 and 7 weeks of age, with nearly half (46%) from 5- and 6-week-old birds. Disregarding the cases from the 1- and 8-week-old birds, the percentage of cases positive for Arkansas and Connecticut IBV, relative to the total cases positive for all viruses, was substantial. Arkansas detections were in the 41% to 62% range, and Connecticut detections accounted for 19% to 37%. It should be noted that adenoviruses were detected only in birds that were 4 to 7 weeks of age.

IBV vaccination program (data available: 200 cases submitted, 122 virus-positive cases, 202 viruses detected; Figure 1D). During 1998, all 8 companies reported vaccination programs that included 1 or more strains of IBV vaccine in combination with the B-1 strain of NDV. Most applied vaccines twice — once by spray in the hatchery when the chicks were 1 day of age followed by a second exposure by water or spray application in the field when the birds were between 14 and 20 days of age. Three of the 8 companies used Massachusetts and Arkansas strains of IBV for these 2 vaccinations throughout the year, while a fourth company started with Massachusetts and Connecticut strains but changed to Massachusetts and Arkansas strains later in the year. Two companies gave the Massachusetts and Arkansas strains throughout the year, while another gave 3 strains — Massachusetts, Connecticut, and Arkansas — in its vaccination program. The remaining company vaccinated using only the Connecticut strain and gave it only once, when their chicks were 1 day of age.

Sixty-seven percent of the virus-positive cases originated from companies vaccinating with the Massachusetts and Connecticut combination strains. Cases received from the other vaccination programs used were Massachusetts and Arkansas strains (27% of all cases), Connecticut only (6%), and 1 case (0.5%) in which all 3 of these strains were used.

These data were examined to determine how many cases that were Arkansas-, Connecticut-, or Massachusetts-positive originated from flocks in which the homologous strain was **not** part of the vaccination program. Of the 94 cases positive for Arkansas, 64 (68%) were from flocks that had not been vaccinated with Arkansas IBV. On the other hand, lower percentages of Connecticut (25%) and Massachusetts (15%) were detected in cases from which the birds had not been vaccinated with these 2 strains, respectively. Based, then, on the numbers of the various viruses detected and especially on the number of viruses detected from flocks not receiving the homologous strain in their vaccination program, the primary cause of this outbreak seems to have been IBV, especially the Arkansas strain and, probably to some degree, the Connecticut strain.

Ultimately, the presence of modified-live IBV vaccines as was used here makes it difficult to accurately determine their role in an outbreak associated with the same virus. This is especially true when the diagnostic regimen that is used employs embryo propagation, the same as is used for vaccine production. Being live, it is possible that the vaccine viruses themselves serve as the primary insult for the outbreak, in which case their detection would be a true positive. It is also possible that vaccine viruses persisting in hosts could be what were detected in the diagnostic procedures, regardless of what the true initiating pathogen was, in which case their detection would be a false positive.

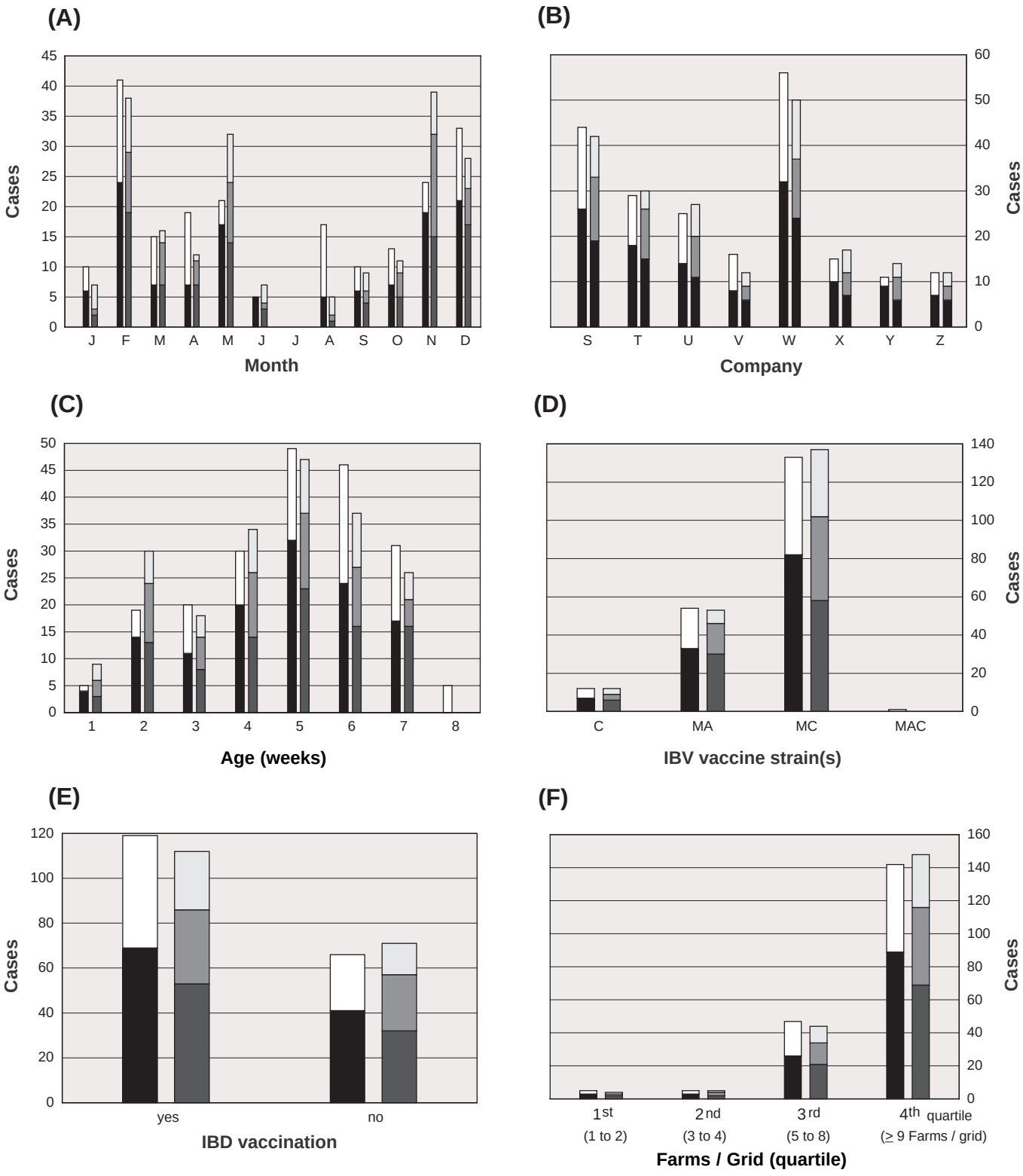

Figure 1. Distribution of cases and viruses isolated from Mississippi broilers during the 1998 respiratory disease outbreak by (A) month received, (B) broiler company, (C) age, (D) IBV in vaccination program, (E) use of IBD vaccination, and (F) farms per grid quartile. The leftmost bar of each paired set shows the number of cases submitted per parameter, with black representing the number of virus-positive cases and white representing the number of virus-negative cases. The rightmost bar of each pair shows the number of virus-positive cases containing the different viruses detected (in varying shades of gray).

Empirical evidence about the role that Arkansas IBV vaccination, in particular, had on this outbreak was also unclear. Those companies vaccinating with the strain expressed their opinion that including Arkansas in their vaccination program afforded some control over the problem. On the other hand, a strong case can be made for the vaccine initiating and fueling the outbreak. The company having the first 2 cases and, coincidentally 1 of the companies with considerable clinical problems, was vaccinating with Arkansas IBV at the beginning of the outbreak and continued to do so throughout the year.

Use of infectious bursal disease (IBD) vaccine (data available: 185 cases submitted, 110 virus-positive cases, 183 viruses detected; Figure 1E). One hundred and nineteen (64%) of these cases were from flocks in which the broilers had received exposure to IBD vaccine. The numbers of virus-positive cases, the number of total viruses detected, and distribution of the various viruses were proportional to the distribution of these cases, regardless of whether they were vaccinated with IBD vaccine or not.

The ratio of cases originating from IBD-vaccinated versus IBD-nonvaccinated flocks was nearly 2:1. Based on this distribution, it is tempting to speculate that IBD vaccination had a significant impact upon the outbreak. However, because the true prevalence of IBD vaccination for the total industry, not just for those cases submitted, is not known, it is not possible to determine whether this is so.

Tracheal condition scores (data available: 182 cases submitted, 109 virus-positive cases, 181 viruses detected). Most (69%) tracheas were received without any detectable alteration in their gross condition (i.e., tracheal score = 0). In general, the number of cases submitted and the number of cases that were virus-positive generally followed the same distribution, that is, those having a tracheal score of 0 >> 1> 2 > 3. The number and types of viruses detected also followed this same general trend. However, a disproportionately large number — 45 (79%) of the 57 cases in which Connecticut was detected — occurred from tracheas having a score of 0. Likewise, 10 of the 11 Massachusetts IBV cases detected occurred in tracheas having a score of 0, with none detected from tracheas having a score higher than 1.

Respiratory lesion scores (data available: 110 cases submitted, 68 virus-positive cases, 112 viruses detected). In a scoring system that ranged from 0 to 3, half of the cases originated from birds that had a lesion score of 2. In decreasing frequency, the remaining cases were in score = 1 (25 cases, 23% of total), score = 3 (22 cases, 20% of total), and score = 0 (8 cases, 7.3% of total). Despite the considerable difference in the number of cases in each of the 4 scores, the relative frequency of Arkansas and Connecticut IBV relative to the total viruses detected within each score remained fairly constant: 43% to 50% for Arkansas and 32% to 43% for Connecticut.

Spatial Analyses

***Location of broiler industry*.** Based on the information supplied by the broiler companies, a total of 8 different companies, some with more than 1 division (i.e., growout complex), were growing broilers in Mississippi during 1998. They identified the location of the industry to 279 of the 36-square-mile grids; however, for all intents and purposes, the bulk (95%) of the industry was located in the southern and central parts of the state and was contained in 266 of the occupied grids (Figure 2A). The remaining part of the industry was located in the northeastern part of the state and, because of its physical separation from the main portion of the industry by both management and distance, was never at risk during the outbreak and is omitted from any further discussion here.

The southern boundary of the at-risk portion of the industry was the horizontal border with Louisiana and extended eastward towards the Alabama state line at about the same latitude. Basically, the western edge of the industry sloped southwest to northeast roughly parallel to Interstate 55. For the most part, the western edge was to the east of I-55; however, there were some occupied grids west of that highway. The eastern edge in its most southern aspects was contiguous with that part of the Alabama broiler industry and some 30 to 35 miles west of the Alabama state line in its more northern aspects. The northern border of the industry was just south of a line connecting the towns of Kosciusko and Louisville, Mississippi.

The more concentrated areas of the industry tended to be within 35 miles north and south of Interstate 20, which runs east to west through the state (Figure 2A). Three particular grids had the highest concentrations in the state, 1 just east of Sebastopol, 1 just northwest of Bay Springs, and the other located some 6 miles east of Laurel. There were also a few areas of increased concentration scattered throughout the more southern aspects of those grids occupied.

The number of companies operating in each grid varied from 1 to 5. Most grids — 157 (59%) out of 266 — were occupied by farms serviced by only 1 company per grid. The number of grids dropped as the number of companies per grid increased: 69 grids (26%) contained 2 companies, 31 grids (12%) contained 3 companies, 6 grids (2%) contained 4 companies, and 3 grids (1%) contained 5 companies.

The distribution of the number of farms per grid was also examined and found to be heavily skewed toward the lower number of farms per grid. Grouping these numbers into the nearest quartile revealed that 107 of the 266 (40%) total occupied grids contained only 1 or 2 farms, 45 grids (16%) contained 3 or 4 farms, 60 grids (22%) contained 5 to 8 farms, and the remaining 67 grids (24%) contained from 9 to 49 farms.

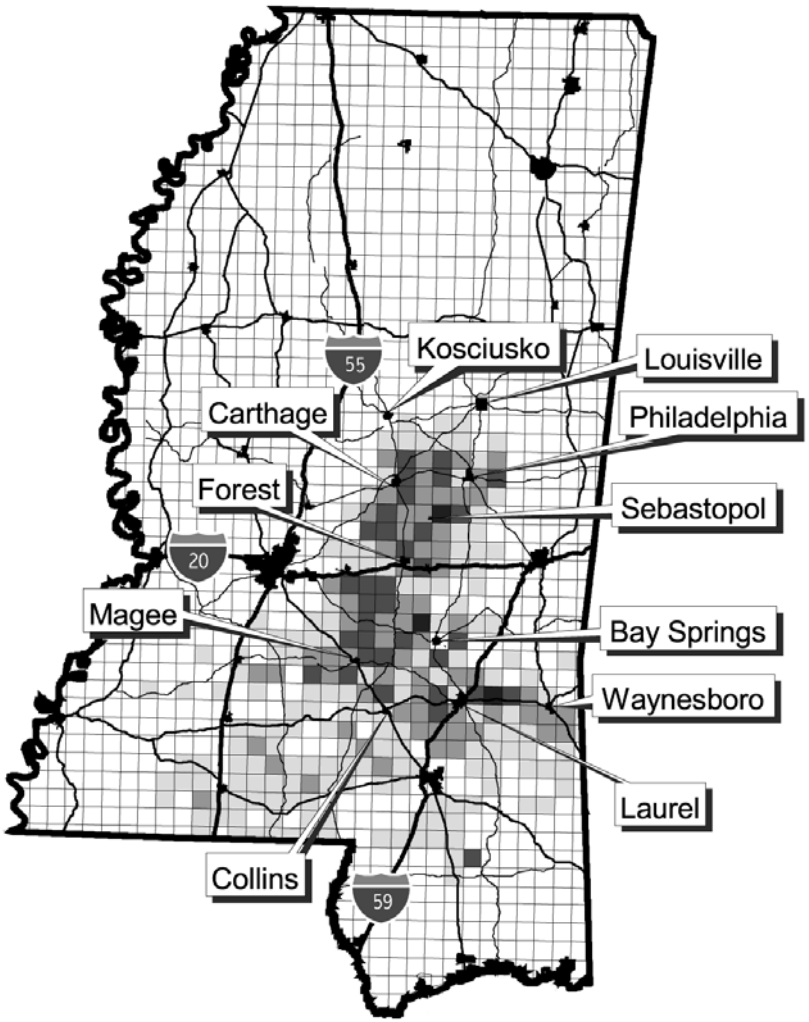

Figure 2A. Map of Mississippi showing 6-square-mile grid overlay system used to locate broiler farms at risk during the 1998 respiratory disease outbreak. Density of broiler farms indicated by shaded grids with darker grids indicating higher concentrations of farms.

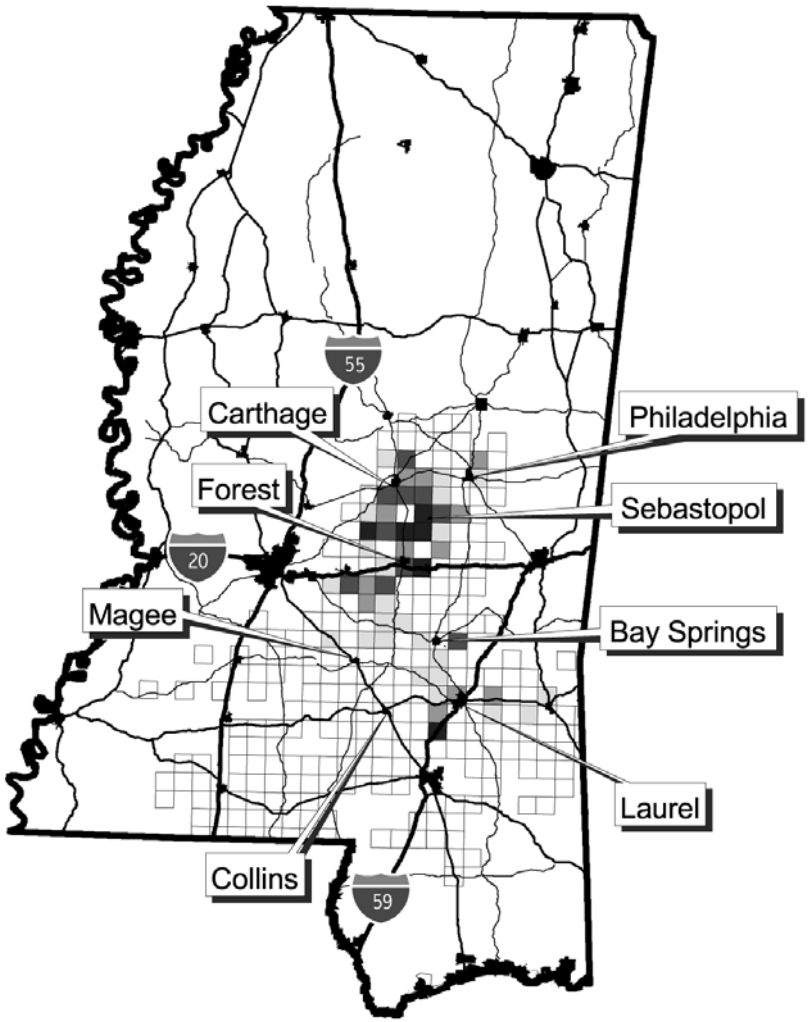

Figure 2B. Grid location of all Arkansas and Connecticut infectious bronchitis (IBV) detections made from Mississippi broilers during the 1998 (January through December) respiratory outbreak. Density of detections indicated by varying shades of gray with the darker colors indicating higher numbers of detections. The remaining at-risk portion of the broiler industry, from which no Arkansas and Connecticut infectious bronchitis (IBV) detections were made, is indicated by clear grids.

Location of outbreak (data available: 201 cases, 121 virus-positive cases, 201 viruses detected; Figure 1F). The farms-per-grid quartiles mentioned above were used to analyze the distribution of cases. Only 5 of these 201 cases were received from the first quartile (1-2 farms per grid); an additional 7 cases were received from the second quartile (3-4 farms per grid), while 47 cases (23%) were received from the third quartile (5-8 farms per grid). Clearly, with 142 cases, the fourth quartile (9-49 farms per grid) represented the largest percentage (71%) of cases submitted.

Chronologically, once established, the Arkansas and/or Connecticut IBV aspect of the outbreak moved fairly rapidly. The first/index case (Arkansas and/or Connecticut IBV) originated from a flock located near Sebastopol. Five days later, the second case (Arkansas IBV only detected) came from a grid in the Forest area, some 12 miles to the south of Sebastapol (Figure 3A). By the end of February, 20 additional cases of Arkansas and/or Connecticut IBV had been detected, involving 18 different grids — including the 2 grids affected in January — and an area measuring 648 square miles. While most affected grids were at a level with or north of Interstate 20 — in and around the Forest, Sebastopol, and Carthage areas — another pocket of 2 grids appeared northeast of Magee (Figure 3B). During May, several positive cases were received from grids to the south, specifically in the Laurel vicinity and 1 grid to the northeast of Magee (Figure 3C). During September, positive cases appeared in a few more grids, most of which were peripheral to those previously affected. This enlarged the number of affected grids in the eastern part of the industry in the Waynesboro area and 1 grid just south of Pelahatchie (Figure 3D). By the end of the year, Arkansas and/or Connecticut IBV were detected in flocks located in a total of 52 different grids (Figure 2B). This represented 19.5% of the 266 grids occupied by this portion of the industry and an area measuring 1,872 square miles. The remaining viruses recovered — Massachusetts IBV, NDV, and adenovirus — were scattered throughout these same 52 grids plus 7 more grids contiguous to them.

Comparing Figure 2B with Figure 2A, it can be seen that the density of positive Arkansas and/or Connecticut IBV detections (Figure 2B) appears to be similar to the pattern of broiler concentration (Figure 2A), with a few notable exceptions. First, the outbreak of these 2 strains was confined to the central and eastern aspects of this portion of the industry, while the extreme southern and western parts were spared, even though these parts contained some areas of increased bird concentration. Also, Arkansas and/or Connecticut detections were noticeably lacking from the heavily populated grid just northwest of Bay Springs, Mississippi.

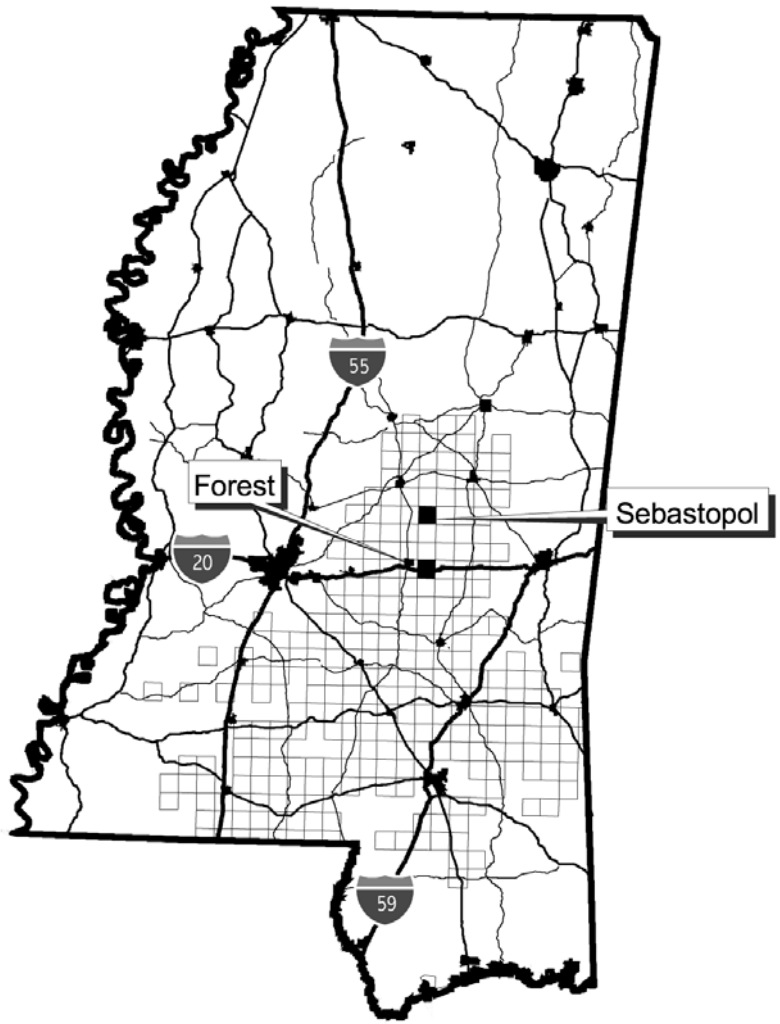

Figure 3A. Grid location of first two Arkansas and Connecticut IBV detections in January 1998 — indicated by darkened grids (clear grids indicate the total industry-at-risk).

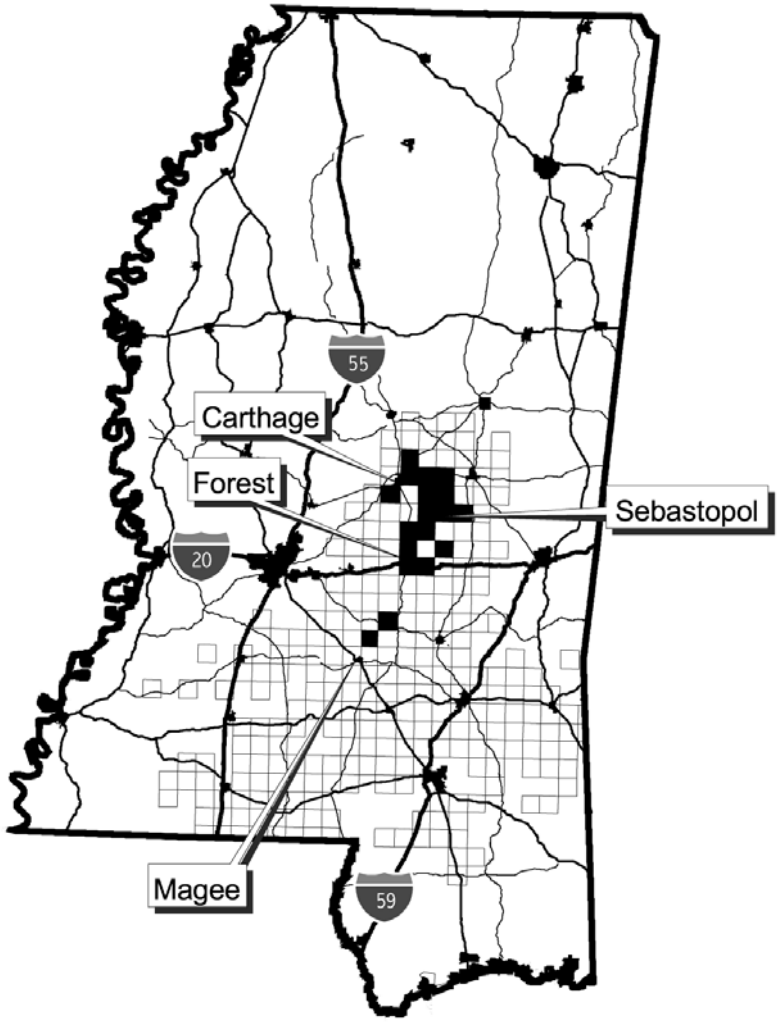

Figure 3B. Progress of Arkansas and Connecticut IBV detections by the end of February 1998 — indicated by darkened grids.

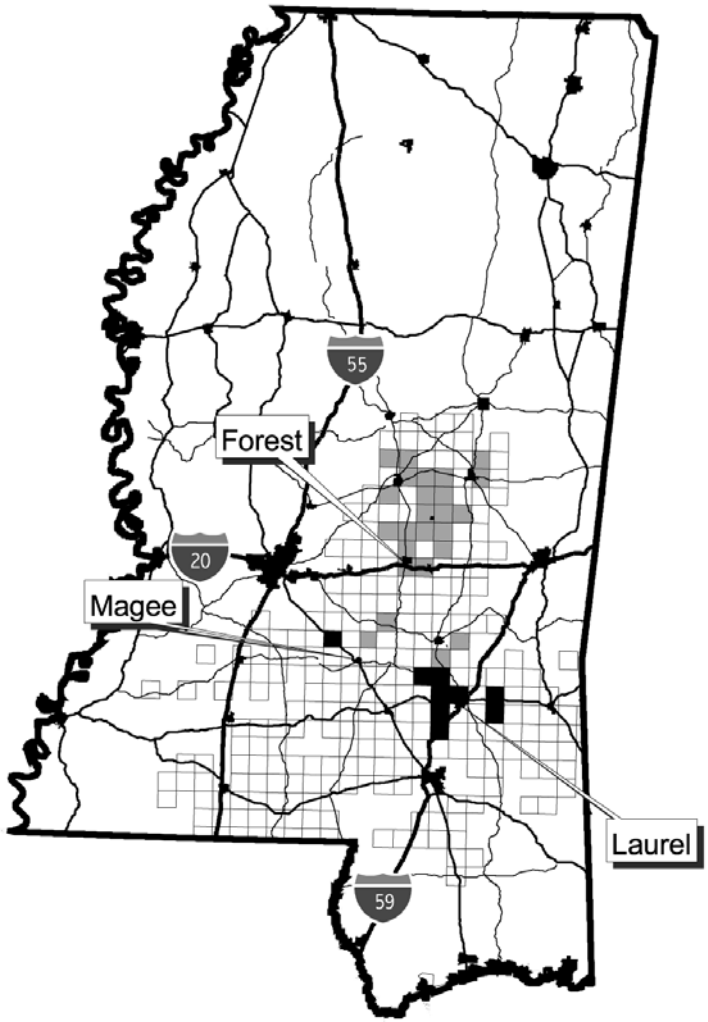

Figure 3C. Progress of Arkansas and Connecticut IBV detections by the end of May 1998. Darkened grids represent May detections, while gray grids indicate earlier detections (January through April 1998).

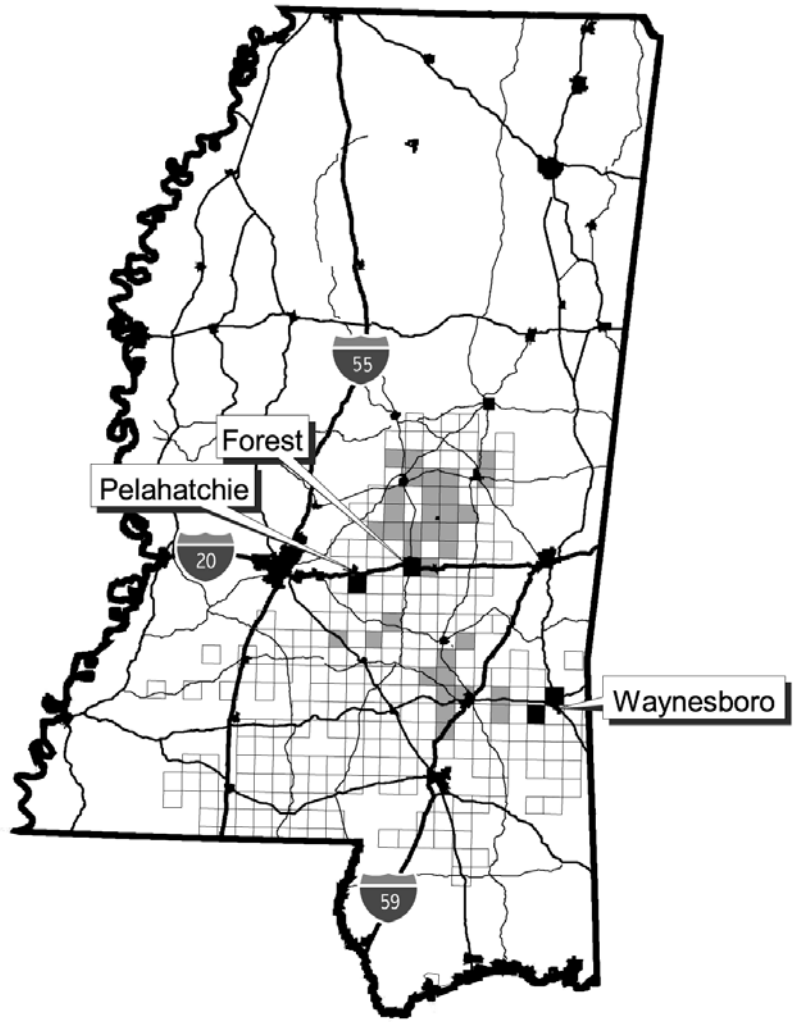

Figure 3D. Progress of Arkansas and Connecticut IBV detections by the end of September 1998. Darkened grids represent September detections, while gray grids indicate detections (January through August 1998).

CONCLUSIONS

1. Based on numbers of viruses detected and on detecting viruses from flocks that had not been vaccinated with homologous strains, Arkansas and, to a lesser extent, Connecticut IBV were responsible for this outbreak.
2. A combination of embryo passages coupled with indirect FA and PCR amplification techniques detected the largest number of viruses.
3. A few cases contained *O. rhinotracheale* and *B. avium*; however, no clinical significance could be attributed to this finding.
4. In general, number of cases received, number of cases virus-positive, and number of viruses detected were proportional to the distribution of cases among the various epidemiological attributes monitored.
5. The greatest number of cases was received during 4 months: February, May, November, and December. Few or no virus-positive cases were received during the summer.
6. Eight different companies were growing broilers in Mississippi during 1998. Although some submitted substantially more cases than others, proportionately all had the same percentage of viruses recovered from their submissions.
7. Because the IBV vaccines used here were based on modified-live viruses propagated in embryonated eggs, the role of these vaccines in an IBV outbreak was difficult to critically evaluate, especially when the diagnostic regimen that was used depended on embryonated egg propagation. However, the number of Arkansas IBV, and to a lesser extent Connecticut IBV, detected from flocks not vaccinated with the homologous strains provided supportive evidence that these 2 strains played a major role in the outbreak.
8. Based on the number of cases originating from IBD-vaccinated farms, the use of that vaccine may have contributed to the outbreak; however, without knowing if this usage prevailed throughout the total industry, the role of IBD vaccination cannot be confirmed.
9. Although the at-risk area occupied by the poultry industry was considerable, cases were received from only about 19% of this area. In general, cases were received from the more concentrated areas within the poultry industry; however, there were many areas of high concentration from which no cases were received.

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ACKNOWLEDGMENTS

The authors express their appreciation to Drs. Danny Magee and Sue Ann Hubbard for professional guidance and assistance. We also thank Elise McKinley, Suzann Tracey, Lana Jones, Debbie Adams, Chau Hong, and Mary Scruggs for technical assistance. Dr. Robert Keirs deserves special recognition for stimulating interest in augmenting our IBV diagnostic regimen to include FA and PCR testing.

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