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## **An Investigation of Variables Associated with Mortality in a Broiler Complex in Mississippi**

Leslie B. Johnson

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An investigation of variables associated with mortality in a broiler complex in  
Mississippi

By

Leslie B. Johnson

A Thesis  
Submitted to the Faculty of  
Mississippi State University  
in Partial Fulfillment of the Requirements  
for the Degree of Master of Science  
in Veterinary Medical Science  
in the College of Veterinary Medicine

Mississippi State, Mississippi

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2019

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A southern Mississippi broiler complex in an area of high poultry density experienced persistent lower livability and growth performance compared with company averages for the state. A retrospective analysis of data from a three-year period (March 2014 through March 2017) was designed using univariable and multivariable statistical analyses to investigate the role of disease, settlement, geographic, and weather variables in broiler mortality. First-week mortality, processing age, average processing weight, genetic line, NDV/IBV vaccination program, and heat index in the last 14 days of the grow-out period were found to be significantly associated with flock mortality in this broiler complex ( $P \leq 0.05$ ). The results of this study should guide future management and disease control strategies aimed at reducing broiler mortality. Additional studies with more diagnostic data and broiler vaccination information are needed to further investigate the relative contribution of disease and vaccination programs to broiler flock mortality.

## DEDICATION

This work is dedicated to the continued collaboration between the Mississippi State University College of Veterinary Medicine Poultry Research and Diagnostic Laboratory and the flourishing southern poultry industry. I would also like to dedicate this project to the future residents of this master's program.

## ACKNOWLEDGEMENTS

First and foremost, I would like to thank my family, my boyfriend, and Dr. Jessica Hockaday for their continued support of me pursuing my dream of becoming a poultry veterinarian. Their dedication and love have made this a rewarding endeavor, and I would not be where I am today without their unwavering encouragement.

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Furthermore, I would like to thank Dr. Jim Watson, State Veterinarian of Mississippi, for providing the geographic information necessary to complete the poultry farm/house density analysis used in my study.

Finally, I would like to thank the poultry company that worked with me throughout this project. They were always willing to help and provide additional information I needed to complete the research, and I am very thankful for the various learning opportunities they afforded me.

## NOMENCLATURE

ADG	Average Daily Gain
AIC	Akaike information criterion
Avg.	Average
DOA	Dead on arrival
FCR	Feed Conversion Ratio
GMT	Geometric Mean Titer
IBD	Infectious Bursal Disease
IBV	Infectious Bronchitis Virus
MM1	Multivariable model 1
MM2	Multivariable model 2
NDV	Newcastle Disease Virus
PCR	Polymerase Chain Reaction
REO	Reovirus
RH	Relative humidity
RR	Relative risk
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SD	Standard deviation
Temp.	Temperature (Celsius)



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## CHAPTER I

### INTRODUCTION

A multitude of factors can affect bird performance throughout the life of a broiler flock. These must be considered by the veterinarians and producers that make decisions regarding flock management. Among these factors are diseases, downtime between successive flocks, farm/house density, age at processing, and seasonal variables (9,26,47,55,58). Broiler flock performance can be measured by feed conversion ratio (FCR), livability, and average daily gain (ADG) (47).

#### **1.1 Background**

This research was initiated by a poultry company with a broiler complex in southern Mississippi that has experienced persistent lower growth performance and livability compared with company averages for the state. The complex experienced several years of increased mortality, decreased broiler performance, and increased airsacculitis condemnations at processing compared with other Mississippi complexes within the company.

##### **1.1.1 Study Objectives**

The overall goal of this research was to investigate the impact of flock specific/settlement, disease, weather, and geographic variables on mortality in a broiler complex in southern Mississippi. The specific objectives of this study were:

1. Collate flock specific/settlement data (including downtime, number of chicks started, processing age, processing weight, first-week mortality, genetic line, year, and broiler vaccination programs), diagnostic/disease data (processing age IBD, IBV, NDV, and REO titers), geographic data (farm/house densities), and weather data (temperature and humidity) for all flocks processed in the complex during the three-year study period (March 2014 to March 2017).
2. Statistically analyze the data to determine the impact of farm specific/settlement, disease, geographic, and weather variables on broiler mortality.

## CHAPTER II

### LITERATURE REVIEW

#### 2.1 Introduction

Previous studies investigated the relationship of broiler performance with a variety of factors, including breeder age, hatchery source, downtime between successive flocks, disease, season, stocking density, weather, age of the houses, litter material, and length of the grow-out period (9,26,47,48,63). The focus of this project is to evaluate the impact of disease (based on processing age IBD, IBV, NDV, and REO serologic titers), commercial poultry farm/house densities (number of farms and houses in 1 km, 5 km, 10 km, 15 km radii), downtime between flocks, processing age, processing weight, first-week mortality, genetic line, year, broiler vaccination programs (NDV, IBV, and IBD), and weather data (average temperature, average heat index, and average humidity for the first 7 days and last 14 days of grow-out) on broiler mortality in a southern Mississippi broiler complex for the three-year study period (March 2014-March 2017).

#### 2.2 Diseases affecting broiler mortality

##### 2.2.1 Infectious Bursal Disease

Infectious bursal disease (IBD) is an economically important immunosuppressive disease of chickens that was first identified in the United States in 1957 as a cause of acute broiler morbidity and mortality (27,36). IBD is caused by a double-stranded RNA virus which is a member of the *Birnaviridae* family that targets the immune system,

particularly the Bursa of Fabricius, and predisposes the host to secondary infections, vaccination failure, increased mortality, decreased performance, and increased processing condemnations (27,49,63). The virus exists in classical and variant forms, though most strains circulating in the United States are of the variant form (63). Infectious bursal disease virus is ubiquitous in the poultry house environment and is resistant to many disinfectants; therefore the disease often reappears in subsequent flocks after a farm has been contaminated (27,40,49,63). Chickens are most susceptible to clinical disease at 3-6 weeks of age, while birds infected at less than 3 weeks of age exhibit subclinical disease with subsequent immunosuppression (19). Diagnosis of disease can be made using virus isolation and RT-PCR in addition to clinical history and the presence of gross lesions in the bursa at necropsy (19). Control of IBD is typically dependent on the use of killed vaccines in breeders to protect progeny from early immunosuppressive infections, and live-attenuated vaccines in broilers (19,20,27,49). Live vaccines may include classic or variant strains of IBD and are classified based on virulence (i.e. mild, intermediate, hot, etc.) (19).

### **2.2.2 Infectious Bronchitis Virus**

Infectious bronchitis virus (IBV) is a highly contagious single-stranded RNA coronavirus that was first identified in the United States in the 1930s (28). The virus has the capacity to affect the respiratory, renal, and reproductive systems of chickens resulting in significant economic losses in poultry throughout the world (4,5). In broilers, IBV infection causes respiratory disease, secondary bacterial and viral infections, increased mortality, poor weight gain and feed conversion, and increased condemnations



at processing (4,11,15). In addition to clinical signs, lesions, and rising antibody titers, IBV diagnosis is made through the use of virus isolation and real time RT-PCR (28). IBV control is attempted through the use of live-attenuated and inactivated vaccines, but this approach is not always effective due to the emergence of new serotypes against which available vaccines may exhibit minimal to no cross-protection (4,13,15,51). The most commonly used vaccinations in the United States are developed from the Massachusetts, Arkansas, and Connecticut serotypes (4,11,21,51).

### **2.2.3 Newcastle Disease Virus**

Newcastle disease, caused by the single-stranded RNA virus, avian paramyxovirus serotype-1 (APMV-1), was first described in England in 1926 (64). It is one of the most important animal diseases worldwide due to the number of animals affected and the economic impact on the poultry industry (7,10,17). The virus is classified into three pathotypes based on disease severity: lentogenic (low virulence), mesogenic (intermediate virulence), and velogenic (high virulence) (17). The most widely accepted means of determining viral virulence is based on the intracerebral pathogenicity index (ICPI) (10). Detection of NDV is typically made through RT-PCR or virus isolation (64). Most outbreaks in broilers in the United States are due to lentogenic strains or from vaccine reactions, resulting in mild respiratory or enteric disease (17,57). Secondary bacterial or viral infections are common complications of infection and lead to increased morbidity (10). Vaccination of broilers through the use of low virulence live vaccines (typically LaSota or Hitchner B-1) is commonly implemented to lessen clinical signs of infection and decrease viral shed (2,7,33,57).

#### 2.2.4 Avian Reoviruses

Avian reoviruses, a group of double-stranded RNA viruses, are ubiquitous in the commercial poultry industry, though most isolates from poultry farms are non-pathogenic (29,44). These viruses cause a wide variety of disease conditions including viral arthritis/tenosynovitis, enteric disease, runting-stunting syndrome, malabsorption syndrome, immunosuppression, respiratory disease, and central nervous system signs (29,44,54). Reovirus infections appear to be prevalent in broiler flocks and economic losses are often the result of lameness, poor performance, and condemnations (14,44). Additionally, depending on the severity of inflammation in the tendons and joints, affected birds may be unable to reach feed and water, resulting in an increase in mortality, mainly as a result of culling (14,44). Diagnosis of reovirus is predominantly made through detection of virus by virus isolation in cell culture or RT-PCR (29,44). Immunization of broiler breeder pullets with live and inactivated vaccines provides the primary means of disease control in broilers through the transfer of maternal antibodies (14,25,29).

#### 2.3 Downtime

Downtime, or the time interval between successive flocks on an individual poultry farm, has been shown to have an effect on commercial flock performance and risk of disease (1,6,12,47). In a study performed by Sasaki *et al.* analyzing flock performance on commercial broiler farms in Japan, flocks that had a short time interval ( $\leq 21$  days) between successive flocks had poorer performance (47). With a shorter downtime, companies are able to increase the number of production cycles per year, but it is critical

to perform thorough cleaning and disinfection between flocks to prevent disease spread to successive flocks (22,47). A study investigating farm-specific risk factors of *Campylobacter* spp. colonization in broilers showed that the highest risk for contaminating a new flock was due to inadequate cleaning and disinfection, as well as insufficient downtime (1). Extended downtime with thorough cleaning and disinfection between flocks has been shown to help eliminate viruses and control diseases (12).

#### **2.4 Farm/house density**

Several studies have been performed analyzing risk factors for disease transmission on broiler farms related to the density of houses or farms in a given area. Previous studies characterizing on-farm sources of *Campylobacter* spp. in broilers suggested that the presence of adjacent broiler farms increases the risk of disease transmission to a new flock (1,34,43). Another study identifying risk factors for *Campylobacter* spp. colonization revealed that the risk of infection increased with increasing numbers of houses on a farm (6). In a study analyzing factors involved in the introduction of Infectious Laryngotracheitis Virus onto broiler farms, closer proximity to and a higher density of neighboring chicken farms was associated with an increased risk (58). Additionally, a study describing the dynamics of an IBD outbreak in Denmark found that the highest risk of disease was observed within a distance of 20 km from the index farm (46). A study identifying risk factors associated with colibacillosis outbreaks in layers demonstrated that an increase in the distance to the nearest poultry farm was associated with a decreased risk (55). Increased distance to the nearest poultry farm has also been associated with a reduced risk of NDV seroprevalence (18). Increased poultry

farm and house density in a given area can allow a disease to spread more effectively through fomites, on vehicles/equipment, and by humans (55,58).

## **2.5 Weather**

Seasonal variations in temperature and humidity have been shown to have an effect on broiler health and production, with worse performance typically occurring in winter months (41). This poorer performance can be attributed to the reduction in ventilation rates that occurs in poultry houses during periods of colder outdoor temperatures (47). However, peak mortality rates tend to occur in summer months as the environmental temperature and relative humidity increase (35,45,56). Other studies have demonstrated that flocks placed in summer months have a higher feed conversion ratio and lower average daily gain, as well as decreased livability (47). Heat stress has a negative effect on broiler performance, resulting in a higher feed conversion ratio and immunosuppression (52). Increasing temperature has also been shown to affect broiler feed conversion and growth after 3 weeks of age, with a greater effect as body weight increases (39). Low relative humidity (RH) can lead to dehydration and poor uniformity, while high RH can result in a greater incidence of footpad dermatitis and hock lesions in addition to impairing the ability of the birds to lose heat via respiratory evaporative cooling (8,30). Insulation of poultry houses, in addition to adequate ventilation, is imperative to reduce the effects of seasonal variations in temperature and humidity (26).

## **2.6 First-week mortality**

Mortality in the first week of the rearing period is typically higher than weekly mortality during the remainder of grow-out (26,61). The first week of a chick's life

represents a transitional period from the hatchery to the broiler farm, and proper management during this period is essential (62). Broiler first-week mortality is an important measure of quality and is related to many factors including breeder age, egg storage length, hatchery, season, housing environment, and bird strain (26,62). Mortality during the first seven days is related to overall broiler performance and mortality during the remainder of the grow-out period and therefore is a critical variable to consider when assessing broiler productivity (9). Chicks that are sick, underweight, stressed, or weak are more susceptible to disease and will not perform to their genetic potential, resulting in economic losses (16,31,53,60,62).

## **2.7 Processing age and weight**

Previous studies by Campe *et al.* (2013) have highlighted the relationship between the length of the broiler grow-out period and increased mortality (3,9). Additionally, outside weather conditions become a more important factor in performance and mortality with longer fattening periods (9). Increasing bird age has also been related to increased risk of diseases, such as *Campylobacter* (37). Additionally, several broiler carcass dispositions/lesions resulting in condemnations at processing, including dead on arrivals (DOAs; birds which arrive dead at the processing plant), ascites, and chronic pathology, have been shown to be positively associated with bird age and bird weight at slaughter (23). Broilers processed at an older age will be exposed to the risk of disease for a longer period of time, and therefore are more likely to be affected by higher mortality rates than broilers processed at a younger age (23). Increasing age at slaughter has been associated

with an increase in the incidence of contact dermatitis, a decrease in the walking ability of birds, and a decrease in thermal comfort (3).

## **2.8 Genetic line**

For the past several decades, the commercial poultry industry has focused on increasing muscle yield and improving feed conversion by genetic selection (24,50,59). However, studies have shown that genetic selection for more efficient growth is positively correlated with mortality (24,50). Faster-growing birds will reach market weights sooner and have less age-related mortality, but may have increased mortality from metabolic diseases such as ascites and musculoskeletal disorders (32,59).

## CHAPTER III

### MATERIALS AND METHODS

#### 3.1 Study area and study period

The study area was an approximately 80 km by 80 km area located in the southern part of the state of Mississippi, United States, incorporating all farms growing chickens for the broiler complex that was the subject of this study. One hundred fifty-eight farms with an average of  $11.87 \pm 4.49$  flocks (mean  $\pm$  standard deviation, SD) processed during the three-year study period (March 2014 to March 2017) were included in the analyses.

#### 3.2 Data collection

Mortality counts, settlement data (including downtime, number of chicks started, processing age, average processing weight, first-week mortality, genetic line, and year), broiler vaccination programs (NDV, IBV, and IBD), and processing-age serology data (NDV, IBV, IBD, REO ELISA geometric mean titers) from the three-year study period were obtained from the broiler company involved in this study.

Weather data, including average temperature (Celsius), average heat index (Celsius), and average relative humidity (percent) for the first 7 days and last 14 days of each flock, were obtained from the nearest airport weather observation station. Geographic data evaluated were numbers of commercial poultry farms and houses within a 1 km, 5 km, 10 km, and 15 km radius from each farm included in the study. The

number of commercial poultry farms and houses within specific radii were calculated using the nearest neighbor function in JMP® 14 (SAS Institute Inc., Cary, NC).

The dependent variable in this study was broiler mortality counts, which were total counts of all chickens that died during the grow-out period of each flock included in the study. Explanatory variables included in the analyses were settlement, diagnostic, weather, and geographic data (Table 3.1). Settlement, weather, and geographic data were available for all 1,865 flocks processed during the study period, while diagnostic data (processing age serology) was only available for 122 flocks processed during this period. Settlement data included downtime (calculated as the number of days between settlement date of a flock and placement date of a successive flock on a single farm), number of chicks started, processing age (days), average weight at processing (kilograms), first-week mortality (percent), genetic line, year, and broiler vaccination programs (Tables 3.2 and 3.3).



Table 3.1 Explanatory variables used in univariable and multivariable analyses to determine association with overall flock mortality counts

<b>Explanatory variable category</b>	<b>Specific explanatory variables</b>	<b>Number of flocks for which data available</b>	<b>Included in multivariable model 1 (MM1)?</b>	<b>Included in multivariable model 2 (MM2)?</b>
Settlement data	Downtime (days) Age at processing (days) Average weight at processing (kg) Week 1 mortality (%) Genetic line Year Broiler vaccination programs (NDV, IBV, IBD)	1,865	Yes	Yes
Geographic data	Number of commercial poultry farms and houses within 1 km, 5 km, 10 km, 15 km radius	1,865	Yes	Yes
Diagnostic data (processing-age serology)	IBD GMT IBV GMT NDV GMT REO GMT	122	No	Yes
Weather data	Average temperature (°C), average heat index (°C), and average humidity (%) for first 7 days and last 14 days of grow-out	1,865	Yes	Yes

Table 3.2 Broiler vaccination program key for live attenuated IBV and NDV vaccines.

<b>Code</b>	<b>Hatchery Vaccine<sup>A</sup></b>	<b>Field Vaccine<sup>A</sup></b>
<b>R1None</b>	IBV Mass <sup>H</sup> (full dose) + IBV Ark <sup>H</sup> (half dose)	None
<b>R2R2</b>	IBV Mass <sup>G</sup> (full dose) + IBV Ark <sup>G</sup> (full dose)	IBV Mass <sup>G</sup> (full dose) + IBV Ark <sup>G</sup> (full dose)
<b>R2R5</b>	IBV Mass <sup>G</sup> (full dose) + IBV Ark <sup>G</sup> (full dose)	IBV Mass <sup>G</sup> (full dose) + IBV Ark <sup>G</sup> (full dose)/IBV Mass <sup>H</sup> (full dose)
<b>R2R6</b>	IBV Mass <sup>G</sup> (full dose) + IBV Ark <sup>G</sup> (full dose)	IBV Mass <sup>H</sup> (full dose)
<b>R3R6</b>	NDV C2 <sup>H</sup> (full dose) + IBV Mass <sup>H</sup> (half dose) + IBV Ark <sup>H</sup> (half dose)	IBV Mass <sup>H</sup> (full dose)
<b>R1R4</b>	IBV Mass <sup>H</sup> (full dose) + IBV Ark <sup>H</sup> (half dose)	IBV GA08 <sup>G</sup> (full dose)

<sup>A</sup>A different superscript indicates a different vaccine manufacturer / tradename

Table 3.3 Broiler vaccination program key for live attenuated IBD vaccines.

<b>Code</b>	<b>Hatchery Vaccine<sup>A</sup></b>
<b>B1</b>	IBD <sup>X</sup> (half dose)
<b>B2</b>	IBD <sup>Y</sup> (half dose)

<sup>A</sup>A different superscript indicates a different vaccine manufacturer / tradename

### **3.3 Preliminary data analysis**

A single master database was created in Microsoft Excel (2013) by compiling settlement, diagnostic, weather, and geographic data. Preliminary data analysis involved basic descriptive statistics in Microsoft Excel (2013), including mean, median, standard deviation, minimum and maximum values, and histograms using PROC UNIVARIATE in SAS® 9.4 (SAS Institute Inc., Cary, NC) to determine the distribution of continuous variables.

### **3.4 Univariable and multivariable statistical analysis**

Mixed model negative binomial regression was used to test associations between the explanatory variables and the dependent variable (broiler mortality counts), using PROC GLIMMIX in SAS® 9.4 (SAS Institute Inc., Cary, NC). The logarithm of number of chicks started was included in the model as an offset. Farm name was included as a random effect to account for variation between different growers.

The strength of associations between broiler mortality counts and the explanatory variables was expressed as relative risk (RR). A RR greater than 1 indicated an increased risk of mortality due to the presence of the explanatory variable while a RR less than 1 indicated a decreased risk. A RR equal to 1 indicated no association between the explanatory variable and mortality counts.

Two separate multivariable models were created, multivariable model 1 (MM1) and multivariable model 2 (MM2). MM1 included settlement, weather, and geographic data for all 1,865 flocks, while MM2 included settlement, weather, geographic, and diagnostic data for the 122 flocks for which diagnostic data (IBD, IBV, NDV and REO GMTs) were available.

The first step in the statistical analysis was univariable analysis to identify variables significantly associated with broiler mortality counts. The diagnostic variables (IBD, IBV, NDV and REO GMTs) were log-transformed to improve convergence and linearity of the models. Each explanatory variable was tested for individual association with the outcome variable; a variable was considered to be significantly associated with mortality counts if the *P*-value was  $\leq 0.05$ . Potential risk factors with a *P*-value  $\leq 0.25$  were retained for further analysis in the multivariable models.

All continuous variables were assessed for collinearity using the CORR procedure in SAS<sup>®</sup> 9.4. Variables found to be collinear, with a correlation coefficient  $> 0.8$ , were further evaluated based on *P*-value, lowest Akaike information criterion (AIC) value, and biological plausibility to determine which variables would be included in the multivariable models.

All candidate variables for each of the two multivariable models, MM1 and MM2, were initially included as explanatory variables in the multivariable models. A manual backward selection process was performed, where the variable with the greatest *P*-value was removed individually until the final model contained only variables with a *P*-value  $\leq 0.05$ . Finally, potential confounding variables were assessed and retained in the final model if adding the removed variable back into the model changed the other coefficients by more than 20%. This process resulted in the inclusion of variables that were not statistically significant, while also changing the significance of other variables in the models.

## CHAPTER IV

### RESULTS

#### 4.1 Preliminary data analysis

For the 1,865 flocks processed over the three-year study period, average downtime was  $13.01 \pm 7.60$  days (mean  $\pm$  standard deviation, SD), mean processing age was  $61.63 \pm 1.53$  days, mean processing weight was  $4.13 \pm 0.19$  kg, average first-week mortality was  $0.71\% \pm 0.20\%$ , and average overall mortality was  $4.34\% \pm 1.66\%$ . Overall mortality increased slightly over the three-year study period, with the highest mortality generally occurring in flocks processed during the summer months of 2014, 2015, and 2016 (Figure 4.1).

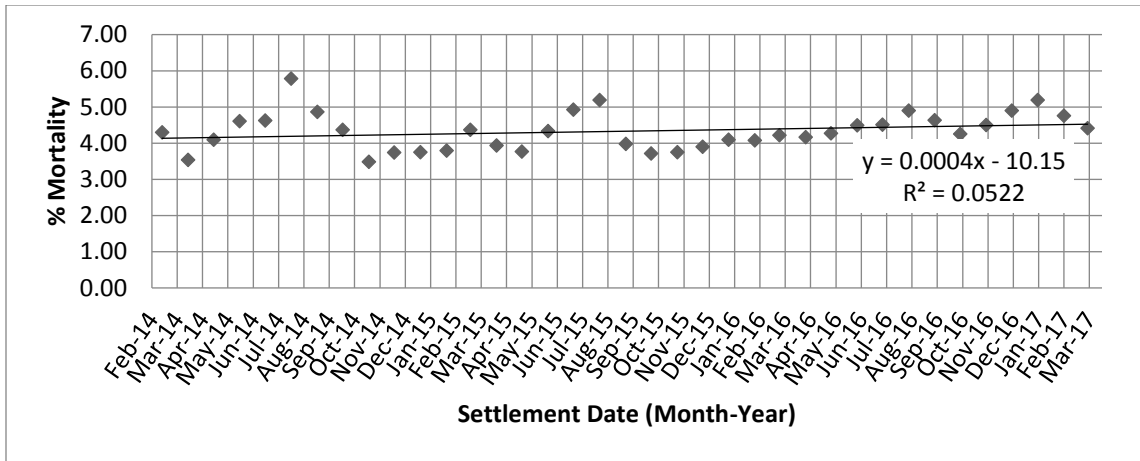


Figure 4.1 Overall flock mortality (percent) over time by settlement date, March 2014-March 2017.

#### 4.2 Univariable analysis

A total of 19 explanatory variables demonstrated statistically significant ( $P \leq 0.05$ ) associations with broiler mortality counts. The screening process for the data set identified 24 variables that had an association ( $P \leq 0.25$ ) with mortality counts and were considered as candidates in the multivariable models (Table 4.1). Although log IBD GMT ( $P = 0.493$ ) and log NDV GMT ( $P = 0.510$ ) did not make the cut-off for consideration in the multivariable models, they were included in MM2 due to special interest in the relationship between serologic titers and broiler mortality.

Table 4.1 Results of univariable analysis to determine variables significantly associated with broiler mortality.

Explanatory variable category	Explanatory Variable	Level or Units	Relative Risk	P-value <sup>A</sup>	
Settlement	Age	Days	1.047	<0.001	
	Average weight	Kg	0.880	<0.001	
	Week 1 mortality	%	1.391	<0.001	
	Downtime	Days	0.992	<0.001	
	Genetic line	A		0.898	<0.001
		B		referent	
	Year	2014		referent	
		2015		0.962	0.006
		2016		1.032	0.029
		2017		1.130	<0.001
	NDV/IBV vaccination program	R1None		0.811	<0.001
		R2R2		0.771	<0.001
		R2R5		0.836	<0.001
		R2R6		0.781	<0.001
R3R6			0.898	<0.001	
R1R4			referent		
IBD vaccination program	None		referent		
	B1		0.975	0.036	
	B2		1.103	<0.001	
Geographic	Farms in 1 km radius		1.026	0.038	
	Houses in 1 km radius		1.006	0.011	
	Farms in 5 km radius		1.004	0.085	
	Houses in 5 km radius		1.001	0.013	
	Farms in 10 km radius		1.002	0.118	
	Houses in 10 km radius		1.000	0.051	

Table 4.1 (continued)

	Farms in 15 km radius		1.001	0.038
	Houses in 15 km radius		1.000	0.014
Diagnostic	log IBD GMT		1.033	0.493
	log IBV GMT		1.051	0.135
	log NDV GMT		1.014	0.510
	log REO GMT		1.061	0.089
Weather	Average Temp. F7	°C	1.004	<0.001
	Average Temp. L14	°C	1.009	<0.001
	Average Heat Index F7	°C	1.003	<0.001
	Average Heat Index L14	°C	1.009	<0.001
	Average Humidity F7	%	1.005	0.001
	Average Humidity L14	%	1.007	<0.001

<sup>A</sup> Variables with  $P \leq 0.05$  were considered significant; variables with  $P \leq 0.25$  were considered as candidates in the multivariable models.

The following continuous variables had a correlation coefficient  $>0.8$ , and were therefore considered to be collinear: average temperature in the first 7 and last 14 days of the grow-out period with average heat index in the first 7 and last 14 days respectively; number of farms in a 1 km, 5 km, 10 km and 15 km radius with number of houses in the same radius; number of houses in a 10 km radius with houses in a 15 km radius; and number of farms in a 10 km radius with farms and houses in a 15 km radius.



Upon further evaluation, based on *P*-value, lowest Akaike information criterion (AIC) value, and biological plausibility, the variables selected for inclusion in the multivariable models were: processing age, average processing weight, first-week mortality, downtime, genetic line, year, NDV/IBV vaccination program, IBD vaccination program, number of farms within a 1 km radius, average temperature first 7 days, average heat index last 14 days, log IBD GMT, log IBV GMT, log NDV GMT, and log REO GMT.

### **4.3 Multivariable analysis**

**4.3.1. Multivariable model 1 (MM1).** MM1 included six effects in the final model that were significantly associated with broiler mortality counts: processing age, average processing weight, week 1 mortality, genetic line, NDV/IBV vaccination program, and average heat index in the last 14 days (Table 4.2). The confounding variables downtime, average temperature first 7 days, number of chicken farms within a 1 km radius, and year were included in the model but were not statistically significant.

Table 4.2 Results of multivariable model MM1, including all 1,865 flocks processed during the three-year study period.

Explanatory variable category	Explanatory Variable	Level or Units	Relative Risk	<i>P</i> -value <sup>A</sup>
Settlement	Downtime	Days	1.000	0.763
	Age	Days	1.061	<0.001
	Average weight	Kg	0.702	<0.001
	Week 1 mortality	%	1.478	<0.001
	Genetic line	A	0.842	<0.001
		B	referent	
	Year	2014	referent	
		2015	0.936	0.083
		2016	0.924	0.068
		2017	0.939	0.256
	NDV/IBV vaccination program	R1None	0.882	<0.001
		R2R2	0.943	0.147
		R2R5	0.936	0.166
		R2R6	0.921	0.278
R3R6		0.984	0.730	
R1R4		referent		
Geographic	Farms in 1 km radius		1.022	0.074
Weather	Average temperature first 7 days	°C	0.997	0.054
	Average heat index last 14 days	°C	1.004	<0.001

<sup>A</sup>Variables with  $P \leq 0.05$  were considered significant.

Evaluation of settlement data showed that an increase in week 1 mortality was associated with a significant increase in overall mortality counts (RR= 1.478,  $P = <0.001$ ) (Table 4.2). A RR of 1.478 indicates that there is a 1.478 times greater risk of overall flock mortality for each one percent increase in first week mortality. Furthermore, as age at processing increased, the risk of mortality also increased significantly (RR= 1.061,  $P = <0.001$ ). However, an increase in average processing weight was associated with a significant decrease in the risk of mortality (RR= 0.702,  $P = <0.001$ ). Genetic line was also significantly associated with mortality counts in this model, with genetic line “A” having a significantly lower risk of mortality than genetic line “B” (RR= 0.842,  $P = <0.001$ ). Downtime, included as a confounding variable, had a RR = 1.000 and  $P$ -value = 0.763 indicating no association between the explanatory variable and mortality counts.

Also included in this MM1 were NDV/IBV broiler vaccination programs and year. All NDV/IBV vaccination programs used by this broiler company during the three-year study period were associated with a decreased risk of mortality compared to the referent R1R4 vaccination program, but this association was only statistically significant for the R1None program (RR = 0.882,  $P$ -value =  $<0.001$ ) (Table 4.2). For the categorical variable year, 2014 was used as the referent as it was the year associated with the highest risk of mortality. Although none of the other years in the study were significantly associated with broiler mortality counts, this variable was included in the model due to confounding.

With regards to geographic variables, an increase in the number of commercial poultry farms within a 1 km radius was associated with an increased risk of mortality,

although this was included as a confounding variable and was not statistically significant (RR= 1.022,  $P = 0.074$ ) (Table 4.2).

Considering the effect of weather variables on mortality counts, there was a significant positive association between average heat index in the last 14 days of the grow-out period (RR= 1.004,  $P = <0.001$ ) and the risk of mortality. However, there was a negative association of average temperature in the first 7 days (RR= 0.997,  $P = 0.054$ ) with the outcome variable, though this was not statistically significant and was included due to confounding.

**4.3.2. Multivariable model 2 (MM2).** The final multivariable model, MM2, included four effects in the final model that were significantly associated with broiler mortality counts: average processing weight, week 1 mortality, genetic line, and average heat index in the last 14 days of the grow-out period (Table 4.3). The confounding variables downtime and number of chicken farms within a 1 km radius were included in the final model but were not statistically significant. Inclusion of these variables in the model also changed the significance of some of the other variables, including processing age and log NDV GMT.

Table 4.3 Results of multivariable model MM2, including 122 flocks for which serologic data was available<sup>A</sup>

Explanatory variable category	Explanatory Variable	Level or Units	Relative Risk	<i>P</i> -value <sup>B</sup>
Settlement	Downtime	Days	1.003	0.135
	Age	Days	1.028	0.082
	Average weight	Kg	0.680	<0.001
	Week 1 mortality	%	1.610	<0.001
	Genetic line	A	0.740	<0.001
	B	referent		
Geographic	Farms in 1 km radius		1.013	0.521
Diagnostic	log NDV GMT		1.038	0.054
Weather	Average Heat Index last 14 days	°C	1.009	0.010

<sup>A</sup> MM2 includes only the 122 flocks for which all four GMTs (IBD, IBV, NDV, REO) were available.

<sup>B</sup> Variables with  $P \leq 0.05$  were considered significant.

Settlement data reflect that an increase in first-week mortality was associated with a significant increase in overall mortality counts (RR= 1.610,  $P = <0.001$ ) (Table 4.3). As processing age increased, the risk of mortality increased (RR= 1.028,  $P = 0.082$ ). However, an increase in average processing weight was associated with a significant decrease in mortality counts (RR= 0.680,  $P = <0.001$ ). Genetic line was also significantly associated with mortality counts in this model, with genetic line “A” having a significantly lower risk of mortality than genetic line “B” (RR= 0.740,  $P = <0.001$ ).

Additionally, in this multivariable model, there was a positive association between downtime, included as a confounder, and mortality counts (RR= 1.003,  $P = 0.135$ ).

The only diagnostic variable included in this model was log NDV GMT. An increase in log NDV GMT was associated with an increase in broiler mortality counts (RR= 1.038,  $P = 0.054$ ) (Table 4.3).

The evaluation of geographic variables shows that an increase in the number of chicken farms within a 1 km radius was associated with an increase in mortality counts (RR= 1.013,  $P = 0.521$ ), although this was not statistically significant and was included in the model due to confounding (Table 4.3).

Considering weather variables, as average heat index in the last 14 days of the grow-out period increased, the risk of mortality also significantly increased (RR= 1.009,  $P = 0.010$ ) (Table 4.3).

The variables that were evaluated in the analyses but not found to be associated with broiler mortality counts in either multivariable model include IBD vaccination programs, as well as IBD, IBV, and REO log GMTs.

## CHAPTER V

### DISCUSSION

The objective of this study was to identify potential risk factors associated with mortality in a broiler complex in southern Mississippi which had experienced decreased livability and bird performance compared to company averages for the state. Previous studies analyzed the association of broiler performance and mortality with a variety of factors; including downtime, weather variables, and length of the grow-out period (9,26,47). In the current study, the effects of disease/diagnostic variables (processing-age serologic titers for IBD, IBV, NDV, and REO), settlement variables (downtime, age at processing, average weight at processing, first-week mortality, genetic line, year, broiler vaccination programs), geographic variables (number of commercial chicken farms and houses within 1 km, 5 km, 10 km, 15 km radii), and weather variables (average temperature, average heat index, and average humidity for the first 7 days and last 14 days of grow-out) on broiler mortality over a three-year study period (March 2014 to March 2017) were investigated.

Variables significantly associated with broiler mortality in both multivariable models, MM1 and MM2, included average heat index in the last 14 days of the grow-out period, week 1 percent mortality, average processing weight, and genetic line. An increase in heat index during the last two weeks of the broiler grow-out period was associated with an increase in overall mortality counts in both models. This finding is

consistent with previous studies which have shown mortality rates increase during the summer months, as temperature and relative humidity increase (35,45,47). These results highlight the importance of adequate ventilation and temperature and humidity regulation, particularly in the last 2 weeks of the broiler grow-out period. The positive association between first-week mortality and overall mortality in both multivariable models underscores the importance of chick quality and good management during the critical first week of a chick's life (9,62).

Average processing weight was significantly negatively associated with mortality counts in both models, indicating that an increase in processing weight was associated with a decrease in mortality counts. The most plausible explanation for this association is that healthy birds will have a higher rate of daily weight gain and will therefore reach processing age with lower death loss. Finally, genetic line was a significant variable in both models, with genetic line "A" having a lower risk of mortality than genetic line "B." Many commercial poultry companies change bird genetic lines over the years and may choose a genetic line that has better performance and feed conversion, but there may be a trade-off with mortality (32,50).

An increase in processing age was associated with an increase in broiler mortality counts in both multivariable models. This likely relates to increased risk of illness (e.g. cardiac failure, heat stress, skeletal problems) as the grow-out period is extended, due to increasing bird weight and increasing stocking density, as well as increased days at risk (24,32,50). It is also possible that flocks which experienced disease associated with higher mortality would be more likely to have lower rates of daily gain, necessitating later processing than healthy flocks.



The R1None NDV/IBV vaccination program was significantly associated with a decreased risk of broiler mortality when compared with the referent NDV/IBV vaccination program, R1R4. This indicates that, in this study, flocks vaccinated with Mass IBV (full dose) and Ark IBV (half dose) vaccines in the hatchery which were not subsequently vaccinated against IBV in the field had lower mortality than flocks which received the GA08 IBV field boost vaccination at approximately two weeks of age. It should be noted, however, that several factors which may have influenced vaccine program performance were not analyzed in this study, including breeder vaccination programs / maternal immunity, IBV field challenge strains, and Marek's vaccines, including recombinant HVT-NDV and HVT-IBD vaccines (which were used in this division during the study period) (38,42). Additionally, it is possible that discrepancies between scheduled vaccination program dates and actual flock vaccination dates, and variations in the lengths of time for which different vaccination programs were applied may have impacted these results.

Another variable that was included in MM2 was log NDV GMT, which had a positive association with mortality counts (i.e. an increase in NDV GMT was associated with an increase in mortality counts). Flocks with higher processing-age NDV titers likely experienced field virus challenge, resulting in respiratory disease and subsequent increased morbidity and mortality (10,17,57).

Variables found to be confounding, and therefore included in MM1, were average temperature in the first 7 days and year. There was a negative association between mortality counts and average outside temperature in the first 7 days in this study, i.e. an increase in temperature was associated with a decrease in mortality risk (RR= 0.997).

During a chick's first week of life, controlling the brooding temperature in broiler houses is imperative as they are not old enough to regulate their own body temperature (62).

Chicks that are too cold will often huddle, and have decreased feed and water consumption, resulting in mortality and poor performance (16,31,53,60,62). The increased risk of mortality during 2014 compared with other years in the study period is likely attributable to the variant Reovirus challenge experienced by this company during that year.

Confounding variables in both multivariable models MM1 and MM2 were downtime and number of commercial poultry farms within a 1 km radius. Although these variables were not statistically significant in either model, they remained in the model due to the fact that they altered other coefficients in the model by a significant amount (more than 20%). Generally, an increase in downtime is associated with a decreased risk of disease and mortality (1,6,12,22,47). However, in these multivariable models, there was no association between downtime and mortality counts in MM1 (RR= 1.000) and a positive association in MM2 (RR=1.003). One plausible explanation for the lack of relationship between downtime and mortality is that more stringent litter management (e.g. windrowing, application of litter amendments) may have been implemented when downtime was reduced. Additionally, downtime may have been reduced on better managed farms or during periods of low disease challenge and increased on farms with a known history of disease. An increase in the number of commercial poultry farms within a 1 km radius was associated with an increase in flock mortality counts in both models. This finding could suggest a role of disease contributing to broiler mortality. Previous

studies have identified the role of farm and house densities in disease transmission, with increased densities associated with increased disease incidence (1,6,7,43).

It should be noted that there are limitations to the data sets used in these analyses. The disadvantage of this retrospective study is that data for certain key variables, notably serologic titers for IBV, NDV, IBD, and REO, were not collected for every flock processed over the three-year period. The only serologic variable that remained in multivariable model 2 (MM2) was log NDV GMT, but this variable did not remain statistically significant after confounding variables were added back into the model. Full serologic data sets were, however, only available for 122 of the 1,865 flocks (6.5%) included in this study, making interpretation of these results difficult. It is suspected that a more significant impact of disease on broiler flock mortality would have been evident if processing-age serologic data had been available for all 1,865 flocks analyzed in this study. As already noted, the finding that an increase in the number of commercial poultry farms within a 1 km radius was associated with an increase in flock mortality supports the likely contribution of disease to mortality in this study. Unlike diagnostic/disease variables, large data sets (1,865 flocks) were available for settlement, geographic and weather variables. It is possible that large sample sizes may have allowed for statistical significance in some variables that may have lower practical significance.

## CHAPTER VI

### CONCLUSION

In summary, this study showed that several commonly recorded production variables (i.e. first-week mortality, processing age, average processing weight, genetic line, average heat index in the last 14 days of grow-out) were significantly associated with overall flock mortality in this broiler complex. The results of this study should guide future management and disease control strategies aimed at reducing broiler mortality, as well as help to set expectations for continued seasonal fluctuation of performance parameters. Future studies with more diagnostic data and broiler vaccination information are needed to further investigate the relative contribution of disease and vaccination programs to broiler flock mortality.

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