

Correlation of Environmental Temperature, Precipitation, and Humidity with Salmonella
Culture Results from Cattle in Virginia

Kwankate Kanistanon

Thesis submitted to the Faculty of the Virginia-Maryland Regional College of Veterinary
Medicine in partial fulfillment of the requirements for the degree of

Master of Science
in
Veterinary Epidemiology

Lorin D. Warnick, Co-Chair
Kevin D. Pelzer, Co-Chair
Jerry R. Roberson

11 March 1997
Blacksburg, Virginia

Keywords: Salmonella, Cattle, Weather
Copyright 1997, Kwankate Kanistanon

Correlation of Environmental Temperature, Precipitation, and Humidity with Salmonella Culture Results from Cattle in Virginia

Kwankate Kanistanon

(ABSTRACT)

Records of bovine samples submitted for salmonella cultures at four regional diagnostic laboratories in the state of Virginia were used to investigate the association of weather conditions and the diagnosis of salmonellosis in cattle. Spearman's correlation coefficients were calculated for the correlations between the monthly number of samples positive for salmonella culture and weather parameters: temperature, precipitation, and relative humidity. Significant correlation coefficients between the monthly average temperature and the monthly number of positive samples were found to be negative in one laboratory ($r_s = -0.38$, $p=0.03$) and positive in one laboratory ($r_s=0.30$, $p=0.02$). The latter correlation coefficient was found between the monthly average temperature and the monthly number of positive samples the following month. The same laboratories that had significant correlation of the monthly number of positive samples and the monthly average temperature also had significant correlation with the monthly average relative humidity ($r_s = -0.39$, $p=0.03$ and $r_s=0.37$, $p=0.004$). The monthly average relative humidity was more highly correlated to the number of positive samples reported in the same month for both laboratories that had significant correlation coefficients. None of the correlations between the monthly precipitation and the monthly number of positive samples were significant ($p>0.05$). The inconsistent directions of correlation coefficients need to be investigated further to find a reason for the discrepancy between regions of the state.

Acknowledgments

There are so many people that greatly helped me and I truly appreciate their help during the past two and a half years that I have been in the United States. Without them I wouldn't have survived the hard work here. Some of the people, excluding my family, friends and sponsor, who help most are listed below:

Dr. Lorin D. Warnick, my major advisor, for giving me a chance to be here, for all the knowledge he shared with me, for study design and support, for being a consultant and for pointing out the beautiful side of Statistics and Epidemiology for me.

Dr. Kevin D. Pelzer, Co-Advisor and **Dr. Jerry R. Roberson** for their advice and this thesis review.

Dr. Robert V. Foutz, Professor, Statistics Department, VA. Tech, for introducing the Durbin-Watson test.

Peter Ammermann, Ph.D. student, Statistics Department, VA. Tech, for statistics advice and ensuring that I knew what I was doing.

Boony Warachan, another statistics consultant, for suggestion and all his statistics books that I never returned.

Dr. Lisa Crofton, director. Harrisonburg Regional Diagnostic Laboratory.

Dr. James Simms, director, **Suzy Trefsgar**, microbiologist supervisor, and

Shelley O brien, microbiologist. Lynchburg Regional Diagnostic Laboratory.

Dr. Joseph Garwin, director, and **Marta Segarra**, microbiologist. Warrenton Regional Diagnostic Laboratory.

Dr. Robert B. Duncan, director, and **William E. Carter**, laboratory supervisor. Wytheville Regional Diagnostic Laboratory.

James Dustin Hux, Dept. of Environmental Sciences, University of Virginia, for relative humidity data.

Special thanks to **Ron Ferguson, Jr.**, for grammar checking, accent correction and much more.

Table of Contents

Abstract.....	ii
Acknowledgments.....	iii
List of Tables.....	vi
List of Illustrations.....	viii
Chapter 1. Introduction.....	1
Chapter 2. Literature Review.....	3
Human Salmonellosis.....	3
Salmonellosis in Cattle.....	4
Review of Statistical Analysis.....	11
Chapter 3. Material and Methods.....	15
Source of Data and Data Collection.....	15
Statistical Analysis.....	17
Chapter 4. Results.....	21
Descriptive Statistics Results.....	21
Correlation Analysis Results.....	30
Regression Analysis Results.....	43
Chapter 5. Discussion.....	47
Chapter 6. Summary.....	51
Appendix 1: Locations of Salmonella Positive Samples and Laboratories.....	54
Appendix 2: Salmonella Isolation Techniques.....	58

References.....	60
Vita.....	69

List of Tables

Table 1.	The percentage of samples positive for salmonella cultures and the serotypes of isolated <i>Salmonella</i>	22
Table 2.	The serotypes of isolated salmonella organisms from studied laboratories and their percentages.....	23
Table 3.	Median values of the monthly number of samples positive for salmonella culture by season.....	24
Table 4.	Median values of the monthly percentage of samples positive for salmonella culture by season.....	24
Table 5.	The number of samples positive for salmonella culture averaged by month and laboratory.....	26
Table 6.	The percentage of samples positive for salmonella culture averaged by month and laboratory.....	27
Table 7.	The descriptive statistics of the monthly number and percentage of the positive samples: the maximum, median and minimum values.....	30
Table 8.	Pearson correlation coefficients between the monthly average temperature and the monthly average maximum, average minimum, highest, and lowest temperature, total precipitation and average relative humidity.....	31
Table 9.	Pearson correlation coefficients between the monthly total precipitation and the monthly average relative humidity.....	31
Table 10.	Spearman correlation coefficients between the monthly number of positive samples and the monthly average temperature and their p-values.....	32
Table 11.	Spearman correlation coefficients between the monthly percentage of positive samples and the monthly average temperature and their p-values.....	33
Table 12.	Spearman correlation coefficients between the monthly number of positive samples and the monthly precipitation and their p-values.....	35

Table 13.	Spearman correlation coefficients between the monthly percentage of positive samples and the monthly precipitation and their p-values.....	36
Table 14.	Spearman correlation coefficients between the monthly number of positive samples and the monthly average relative humidity and their p-values.....	37
Table 15.	Spearman correlation coefficients between the monthly percentage of positive samples and the monthly average relative humidity and their p-values.....	38
Table 16.	Spearman correlation coefficients of the monthly number of positive samples and the weather at the Harrisonburg laboratory with and without the data of 1994.....	41
Table 17.	Spearman correlation coefficients of the monthly percentage of positive samples and the weather at the Harrisonburg laboratory with and without the data of 1994.....	42
Table 18.	Regression analysis of the monthly number of salmonella positive samples at the Harrisonburg laboratory.....	43
Table 19.	Regression analysis of the monthly percentage of salmonella positive samples at the Harrisonburg laboratory.....	44
Table 20.	Regression analysis of the square root of the monthly number of salmonella positive samples at the Warrenton laboratory.....	44
Table 21.	Regression analysis of the square root of the monthly number of salmonella positive samples at the Wytheville laboratory.....	45
Table 22.	Regression analysis of the natural log of the monthly percentage of salmonella positive samples at the Wytheville laboratory.....	46

List of Illustrations

Figure 1.	Epidemiology of salmonellosis in adult cattle.....	8
Figure 2.	The median values of the monthly number of samples positive for salmonella culture by season.....	25
Figure 3.	The median values of the monthly percentage of samples positive for salmonella culture by season.....	25
Figure 4.	The temperature averaged by month for each laboratory.....	28
Figure 5.	The relative humidity averaged by month for each laboratory.....	29
Figure 6.	The total precipitation averaged by month for each laboratory.....	29
Figure 7.	Association of Harrisonburg's monthly number of samples positive for salmonella culture with the monthly average temperature.....	34
Figure 8.	Association of Harrisonburg's monthly percentage of samples positive for salmonella culture with the monthly average temperature.....	34
Figure 9.	Association of Harrisonburg's monthly number of samples positive for salmonella culture with the monthly average relative humidity.....	39
Figure 10.	Association of Harrisonburg's monthly percentage of samples positive for salmonella culture with the monthly average relative humidity.....	39
Figure 11.	Locations of studied laboratories and weather stations.....	54
Figure 12.	Location by county of bovine samples positive for salmonella culture at the Harrisonburg, Lynchburg, Warrenton, and Wytheville laboratories.....	55
Figure 13.	Virginia milk cows: Number on farms, January 1, 1994.....	56
Figure 14.	Virginia beef cattle: Number on farms, January 1, 1994.....	56
Figure 15.	Virginia cattle (beef and dairy): Average number on farms, 1991-1994.....	57

Chapter 1.

Introduction

The genus *Salmonella* is a member of the family *Enterobacteriaceae*.⁴⁹ They are facultatively anaerobic, gram negative, short rod-shaped, and usually motile bacteria that are commonly found in soil and water as fecal contaminants.⁷⁸ *Salmonella* has one species called *enterica* which is subdivided into six subspecies,⁴⁹ *enterica*, *salamae*, *arizonae*, *diarizonae*, *indica*, and *houtenae*.¹⁰ Serogroups of *Salmonella* (e.g., B, C, D, and E.) are identified based on the somatic (O) antigen while serotypes are identified on the basis of combination of O, and flagellar (H) antigens.⁴⁹ *Salmonella* organisms are commonly called by the genus followed by the serotype (e.g., *Salmonella typhimurium*). There are over 2,200 recognized salmonella serotypes. Although all recognized serotypes are pathogenic,⁴⁴ less than 50 serotypes are epidemiologically important.¹⁰ The organism can cause disease in both humans and animals. The major route of infection is fecal-oral. The transmission cycle begins either from human patients with salmonellosis who excrete the organism in feces, or infected animals shedding bacteria in feces and milk. Another source of *Salmonella* is waste material from slaughter houses that leaks into the environment. *Salmonella* from these sources may accidentally contaminate human and farm animal food or water sources. When humans and animals ingest salmonella contaminated materials they may show signs of illness or become asymptotically infected. In either scenario, *Salmonella* may be excreted into the environment. Infected wild birds and rodents are potentially important reservoirs of salmonellosis in farm animals.^{2,83} There are numerous pathways in the cycle involving mechanical and biological vectors. Proposed conditions to reduce the risk of salmonellosis in humans are: 1) having salmonella-free livestock, 2) preventing contamination of meat during slaughter and processing, and 3) prevention of salmonella contamination in the environment.⁵⁹ Reducing the occurrences of salmonellosis in farm animals can be achieved by the combination of good management, hygienic maintenance, and vaccination.⁵⁵ Currently, there are many studies focusing on the epidemiology of salmonellosis in livestock.

To control salmonella infection in humans and animals, many approaches have been considered. One of which is to understand the epidemiology of salmonella infection. This study has been conducted to provide researchers and practitioners information concerning the possible effects of weather on the occurrence of salmonellosis in cattle. This information will result in awareness of the relationship of weather as a risk factor and a potential confounding variable that should be controlled in future studies.

Objectives

The goal of this study was to provide information on the relationship between weather parameters and the occurrence of salmonellosis in cattle. The specific objectives of the study were:

- 1) To provide descriptive information on the laboratory diagnosis of salmonellosis in cattle in Virginia.
- 2) To determine the association between the number of bovine samples positive for salmonella culture in Virginia diagnostic laboratories and weather parameters in Virginia. Selected weather parameters examined were temperature, precipitation, and relative humidity.
- 3) To determine the association between the percentage of salmonella positive bovine samples and selected weather parameters in Virginia.
- 4) To develop prediction equations for estimating the number and percentage of positive samples relative to the weather parameter values for each laboratory studied.

Chapter 2.

Literature Review

Human Salmonellosis

An important human health problem is foodborne disease caused by *Salmonella*. The incidence of salmonella infection in humans has been increasing during the last forty years in the United States.⁹¹ In addition, in 1990 the percentage of patients infected with antibiotic resistant salmonella organisms was higher than in 1980.⁴⁵ The estimated annual number of cases of human salmonellosis in 1987 was 2 million with 1,000 associated deaths.⁷⁰ Cullor reported that among foodborne diseases, approximately 2 million cases (second in incidence) and 900-2000 deaths (leading cause of mortality) caused by *Salmonella* occurred in the United States during 1992.¹³ The economic impact of salmonellosis was the highest among foodborne illnesses caused by microorganisms in the United States.⁹² The annual cost of human salmonellosis (non-typhoid) was estimated to be 4 billion dollars while the annual total cost of foodborne illness in the U.S. was 8.4 billion dollars.⁹²

There are two forms of human salmonellosis; typhoid and non-typhoid. Typhoid fever (enteric fever) is caused by *S. typhi* which appears to cause disease only in humans.⁴⁴ The reported occurrence of typhoid fever in the United States has decreased from 5,500 in 1942 to below 500 currently.³² Presently, it is nearly eliminated in most industrialized countries. In contrast, the reported non-typhoid cases in the U.S. during the 1970s to late 1980s increased and *S. typhimurium* and *S. enteritidis* were the most common isolates (each 20.5% of outbreaks).⁹¹ Clinical signs of non-typhoid salmonellosis include abdominal pain, nausea, and watery diarrhea and is usually self-limiting within about five days of onset of the disease.¹⁶ The incubation period of non-typhoid salmonellosis is about 8-72 hours after consumption of contaminated foods.¹⁶ Duration and severity are dependent on infective serotypes and individual susceptibility.²²

The major source for human salmonellosis is farm animals, which may frequently be carriers of the organisms.⁵⁹ Transmission of salmonella infection to humans occurs most often by ingestion of salmonella contaminated foods, most of which are foods of animal origin.⁹¹ Among identified food vehicles, beef is the most common food vehicle; being identified in 11.2% of outbreaks; followed by chicken 8.8% then eggs 5.3%.⁹¹ Another study using the DNA fingerprinting technique demonstrated that the main sources of human salmonellosis were swine and cattle;⁵⁷ however, the study was done in Italy whereas the previously mentioned study was performed in the U.S. Interestingly, other studies indicated an association between people who developed salmonellosis and their farm animals that were infected with *S. typhimurium*.^{24,96}

Salmonellosis in Cattle

Bovine salmonellosis may present as an acute or chronic disease in individual animals. It may also present as an epidemic or endemic disease in a herd. The two most commonly isolated serotypes in the U.S. are *S. typhimurium* and *S. dublin*.^{71,93} Transmission among animals may occur by eating salmonella contaminated feed.⁴⁰ The infective oral dose in a healthy calf is about 10^6 - 10^{11} cells.¹⁰⁹ The preferential sites for salmonella organisms to penetrate after being consumed are the ileum and cecum.⁶⁹ The organisms are more likely to adhere to the Peyer's patch follicle-associated epithelium than to the villous enterocytes.⁹ *Salmonella* invade the lamina propria where they are engulfed by macrophages.⁶⁹ Some of the invasive salmonella cells can survive phagocytosis by macrophages and thereafter replicate inside large vacuoles and destroy the macrophages.⁴⁶ Cattle with salmonella infections develop clinical signs ranging from mild illness to toxemia and death; death occurring within two to five days after onset of clinical signs.⁷⁹ Severity of clinical signs depends upon the age of the animal, infective dose acquired, and serotype of the bacterium. Host adapted *Salmonella* (*S. dublin* in cattle) are likely to cause a septicemic syndrome; while non-host adapted *Salmonella*, *S. typhimurium*, *S. agona* and *S. montevideo*, for example, tend to cause an enteric syndrome.²⁹ Signs of septicemia include fever, malaise, pneumonia, icterus, toxemia, central nervous system disturbance, and other symptoms depending on affected organs.²⁹ Spontaneous abortions often occur in late gestation and are associated with pyrexia, endotoxemia, bacteremia and uterine infection.⁷⁹ The serotype that is most frequently involved in bovine abortion is *S. dublin*.⁵⁴ Cattle with the enteric syndrome usually develop diarrhea, dehydration and, in severe cases, diarrhea with mucosal detritus in the feces.^{29,88} Significant lesions can be found in the alimentary tract, mesenteric lymph nodes, liver, and spleen.⁸⁸ In the small intestine there is a change in the villi and enterocyte shape as well as invasion of neutrophils into the lamina propria, crypts and intestinal lumen.³³ In adult animals, acute enteritis is the most common sign. The case fatality rate can be as high as 75% if treatments are not given in the early stages.⁶⁴ Cows infected with *S. dublin* may produce congenitally infected calves which are often stillborn.¹⁰⁹ Calves one to three weeks old often have the enteric form, while septicemic salmonellosis occurs more often in one to four month old calves.² The mortality and morbidity rates in calves are very high in outbreaks that develop within two weeks after new animals arrive on the farm.⁷ The rates are reduced if the outbreak occurs six weeks after arrival to the unit.⁷ Calf deaths usually occur after five to seven days if the calves are not treated.³⁹ A California survey showed that dairy farms with *Salmonella* had 3.8 times higher calf mortality rates than dairies that did not have *Salmonella*.⁶⁰ Although *Salmonella* is not the most common enteropathogen isolated from diarrheal calves^{33,50} and newly arrived calves,⁹⁴ the disease is costly. The major cost of an outbreak is associated with mortality.⁶⁵

Artificial aerosol exposure of *S. typhimurium* can cause infections in mice, thus airborne transmission in other animals may be possible.⁹⁹ A field observation showing calves in individual pens were infected suggested the possibility of aerotransmission.¹⁰⁹ After recovering from clinical signs, animals may become carriers resulting in endemic disease within the herd.^{27,85} Intramammary infected cows may excrete *S. dublin* between

10¹ to 10⁶ colony forming units in one ml. of milk for six months after the initial infection.⁸⁵ Isolation of *S. dublin* from fecal and yard slurry samples of an infected herd showed a persistence of the organism during a three year period.¹⁰⁶ *Salmonella typhimurium* may persist in the environment for fourteen months⁵¹ and for three to five years in cattle herds.²⁸ Whenever the balance of the intestinal flora is changed and competitive microorganisms are reduced, *Salmonella* in the intestine may increase in number resulting in clinical disease because it is considered an opportunistic pathogen.⁸³

Diagnosis of Salmonellosis in Cattle

Bacterial culturing of feces and tissues are commonly used for confirming a diagnosis.⁸⁷ The success of a bacterial culture depends on the *Salmonella* serotypes, number of organisms in the sample, frequency of shedding, type of collected samples and culture techniques.³⁸ Conventional culture methods are costly, elaborate and require 72-96 hours before tests can be interpreted;³⁸ however, there are some new cultural methods that require less time but are equally as diagnostic. A 24 hour screen, which is a new technique that combines bacterial culture and serologic testing together developed by Cherrington and Huis In't Veld, can detect viable salmonella cells in feces with about the same accuracy as classical isolation procedures, but requires less time (two compared with five working days).⁸ A rapid culture method introduced by Davies and Wray gives better sensitivity using less media and time than the conventional method for identification of *Salmonella*.¹⁸ A study evaluated the following commercial media for the detection of *Salmonella*; Brilliant Green Sulpha Agar, Bismuth Sulphite Agar, Hektoen Enteric Agar, Xylose Lysine Deoxycholate Agar, EF-18 Agar and Rambach Agar. Bismuth Sulphite Agar and Rambach Agar rated the best for overall performance.⁹⁷ Another study also recommended Bismuth Sulfite Agar because of its high selectivity and saccharide-independent properties.¹⁷ Rectal mucosal cultures yield more salmonella isolations than fecal cultures⁶¹ and fecal cultures yield more isolations than nasal secretion and buffy coat cultures.⁵⁸ Fecal samples from calves are more likely to yield positive results than samples from adult cattle.^{38,58} A tentative necropsy diagnosis in cases of negative salmonella cultures can be based on mucosal ulceration and necrosis with vascular thrombosis of the large intestinal mucosa.⁸⁷

Cattle that are *S. dublin* carriers can be identified immunologically by using an indirect Enzyme-linked Immunosorbent Assay (ELISA) which measures IgG antibodies in milk or serum.^{38,86} A logistic regression model was developed to predict *S. dublin* carrier status from serum and milk IgG and IgM titers to the organisms and from a ratio of IgG to IgM.⁸⁶ False-positive ELISA results, due to cross-reaction with other organisms, can be eliminated by using combinations of highly specific monoclonal antibodies.⁵ A commercial ELISA kit has 69% test sensitivity and 97% test specificity; additionally, the test kit takes 1-3 days less time for diagnosis compared to cultures.⁶³ An O-antigen (O:1,4,5,12) -based ELISA has a herd test sensitivity of 92% and a herd test specificity of 100% for detection of antibodies against *S. typhimurium*.³⁷ Some cows may have high ELISA titers in milk or serum, but post mortem tissues are cultured negative for *Salmonella* because of vaccination, recent infection, or recovery after infection.⁸¹ Hence, the ELISA is useful for identifying exposed animals in a herd, but other methods

are needed to differentiate vaccinated and recovered animals from infected and carrier animals. The antiglobulin test (AGT) is capable of differentiating between *S. dublin* infected cattle from vaccinated cattle because the infected animals are more likely to have high AGT titers.¹⁰⁷ A cutaneous delayed hypersensitivity test has been developed for detecting cattle that are systemically infected with *S. dublin*; however, it is not clear if recovered animals have positive test results as well.¹ The cutaneous delayed hypersensitivity reaction lasts longer than the serum agglutinin response, so the skin test may be more useful than the AGT for detecting *S. dublin* carrier animals.¹ A complement fixation test had a low specificity in detecting *S. dublin* infected cattle as it showed serological activity to other serotypes of *Salmonella*.¹⁰⁸ Although serologic tests have an advantage in that they require less time than bacterial cultures, bacterial cultures are still necessary for serotyping and screening antimicrobial sensitivity.

Enrichment Broth Cultivation-PCR Procedure, which combines a short cultivation procedure with a *Salmonella*-specific PCR-hybridization assay, is a new method used for identifying salmonella organisms. The assay is as sensitive and specific as culture methods for detection of *Salmonella* in clinical samples and identifies the organisms more rapidly than culture methods.⁹⁰ Another advantage of this technique is that samples containing few numbers of organisms that would result in negative microbial cultures will be identified as positive.⁹⁰ It can detect as few as 9 colony forming units of *Salmonella* in pure culture and with as little as 300 femtograms of purified chromosomal DNA.⁹⁰

Treatment

The goals of treating cattle with salmonellosis are to maintain fluid volume, electrolytes, acid-base balance, and control bacteremia and septicemia by the administration of antimicrobial agents.⁶⁹ Fluid therapy centers on use of an alkalinizing solution containing polyionic electrolytes, the dosage being dependent on the degree of dehydration.⁷ In severe cases, metabolic acidosis, electrolyte deficiencies, and hypoproteinemia are likely to be present and must be concurrently treated.⁷ The objective of antimicrobial therapy in salmonellosis is not to eliminate the organism from the gut or to eliminate the diarrhea but to eliminate systemic infection.⁷⁴ Antibiotic sensitivity testing is necessary as *Salmonella* are resistant to many currently available antibiotic treatments. *Salmonella typhimurium* 204C often shows multiple antibiotic resistances.⁷⁹ Gentamicin and sulfonamides were very effective for *Salmonella* isolated from cattle.⁷⁴ In an outbreak of *S. dublin* among dairy calves, a bolus containing sulfadiazine 1.0g. and trimethoprim 0.2g. was given to the calves twice daily for six days resulted in clinical improvement of affected calves within three to four days after treatment.⁷⁷ However, parenteral treatment appears to be more effective than oral treatment.⁶⁹ Amoxicillin and trimethoprim, given orally, are suitable for infected veal calves.³⁰ A single dose of recombinant bovine interferon - α_{11} 5.0 mg./calf, injected intramuscularly, reduces the degree of septicemia in infected calves.⁶² Once an outbreak occurs, the following rules should be applied: 1) separate sick and healthy animals, 2) be careful of cross contamination from carcasses and waste to healthy animals, and 3) no new animals should enter the herd.⁷

Vaccine and Prevention

Preventive measures for salmonellosis in cattle are based upon avoiding introduction of the organism into herds and limiting the spread of infection.⁷⁹ Some recommended preventive protocols include:

- 1) testing all cattle and culling carriers
- 2) testing and quarantining purchased replacements
- 3) feed only salmonella-free feed
- 4) isolate sick animals
- 5) control rodents and prevent wild animals from accessing cattle feed
- 6) any contaminated materials, such as contaminated trucks and waste should not be allowed near animals or feed
- 7) avoid overuse of antimicrobials which may promote bacterial resistance
- 8) vaccinate cattle⁸³

Vaccines have been widely used to control salmonellosis in cattle. The properties of an ideal salmonella vaccine are: 1) capable to stimulate secretory IgA, serum antibody, and cell-mediated immunity and, 2) capable to protect animals against all salmonella serotypes.⁴⁴ Live attenuated, orally-administered vaccines are the most successful in preventing the disease due to the ability to stimulate better cell-mediated immune response compared to killed vaccines.¹⁰ Killed vaccines or bacterins do not induce a mucosal or cellular immune response, and the humoral immunity produced does not last long.¹⁵ The amount and duration of salmonella shedding by calves can be decreased by using orally administered live vaccines.⁵⁵ Killed salmonella bacterin may not induce antibodies against salmonella lipopolysaccharide in calves younger than 12 weeks old;⁷² while the *aro*⁻ *S. dublin* live vaccine can induce a response in calves 7 weeks old.⁸² The same *aro*⁻ *S. dublin* vaccine was confirmed by another study to be effective for mortality protection.⁸⁰ Two killed salmonella vaccines, an oil-in-water emulsion and a modified bacterin, failed to protect calves from a virulent *S. typhimurium* challenge.³ A commercial combination of *E. coli*, *Salmonella* and *Pasteurella* vaccine failed to alter mortality and morbidity rates in Holstein/Friesian calves.⁶⁶

Epidemiology of Bovine Salmonellosis

The prevalence of bovine salmonellosis has been reported by researchers from various areas in the United States. A survey on bulk tank milk from eastern Tennessee and southwest Virginia found *Salmonella* in 8.9% of milk samples.⁷³ In California, a study reported that 73% of non-vaccinated herds had at least one cow with antibodies against *Salmonella* while the percentage in vaccinated herds was 89%.⁸⁴ The rate of fecal shedding in culled dairy cows in Washington state was 0.46%.²⁶ The prevalence of salmonella infection estimated from fecal cultures of neonatal dairy calves in Ohio was 2.2%.⁴³ The true prevalence of salmonellosis in cattle is believed to be higher than the rate reported based on fecal cultures because *Salmonella* are excreted intermittently. *Salmonella* carriers may shed the bacteria in feces only 3-4% of the time.⁸⁴ Another study found that carrier cows shed *S. dublin* in 3.5% of fecal samples and 2.5% of milk samples

while carrier calves shed the bacteria in 17.3% of fecal samples.³⁸ Gay and Hansaker suggested that herd performance and absence of clinical salmonellosis do not indicate that dairies are free of *Salmonella*. They reported that 76% of environmental samples and 48% of fecal samples were salmonella positive two years after an outbreak.²⁷

Many studies have provided information on reservoirs of cattle salmonellosis. Markets, transport vehicles, and dealers are important sources of infection for calf-rearing farms.^{104,105} Calves that were transported after being purchased were more likely to excrete *Salmonella* in feces;¹¹ consequently, the incidence of salmonellosis on farms increased and the maximum number of excretors occurred approximately three weeks after arrival of new calves.^{35,103} A pattern of bovine salmonellosis is shown in Figure 1.

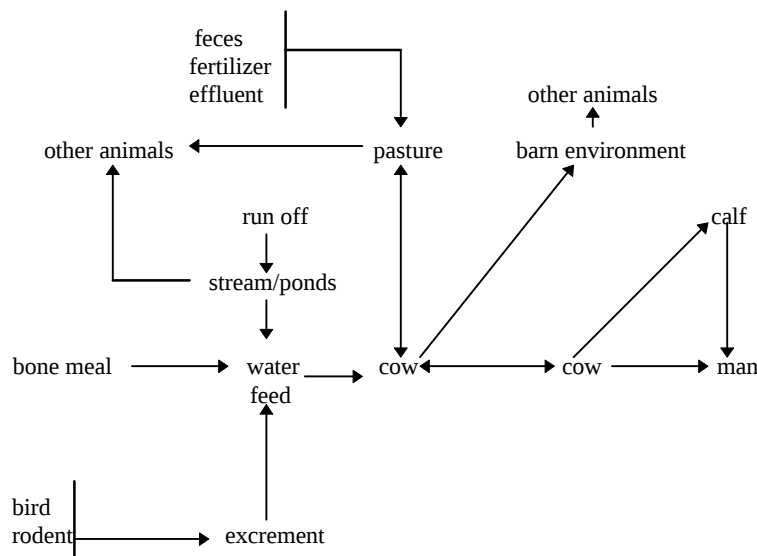


Figure 1. Epidemiology of salmonellosis in adult cattle (Source: McDonough, P. L. 1986. Epidemiology of bovine salmonellosis)⁴⁸

Besides a long persistence in the environment and carrier animals, *Salmonella* can live in many wild carriers such as birds and rodents which potentially serve as reservoirs or physically spread the infection.² *Salmonella* persist in wild bird droppings for four weeks.¹⁹ These factors contribute to the difficulty of preventing outbreaks of salmonella infection.

Risk factors for salmonella infections in cattle are overcrowding, contaminated feed and water, improperly treated farm waste, newly purchased calves, and wild birds.⁷⁹ Neonatal calves are at greater risk of developing salmonellosis than adult cows⁴⁸ because their abomasums lack protective acidity and there is no competing flora in the gut.² Factors that cause animals' stress such as pregnancy, parturition, transportation, changes in diet, and lack of food or water increase the chance of acquiring the disease or shedding the organisms.⁷ *Salmonella* diarrhea in adult dairy cows occurs more often in high producing fresh cows, cattle with displaced abomasums, and cows with other health problems.⁸³ Animals' susceptibility to salmonellosis is increased by concurrent

parasitism, ketosis, metritis, mastitis, cystitis, hypocalcemia, pneumonia, viral infections, dietary changes, water deprivation, and stress factors such as freezing or wet weather.⁴⁴ Liver flukes had been considered as a risk factor since they may alter the balance of intestinal microorganisms, but a recent study showed that liver flukes did not have any significant role in the development of salmonellosis.⁵³ A case-control study revealed that the presence of wild geese was associated with an increased chance of a farm experiencing an outbreak of salmonellosis.⁹⁸

Effect of Weather Conditions on the Occurrence of Salmonellosis in Cattle

In order to evaluate risk factors, prevention and treatment programs, confounding variables that are related to risk factors and that may affect the incidence of the disease should be controlled. A potential confounder is the weather but there have been no studies addressing the effects of weather on the incidence of salmonellosis in cattle. The fact that observing occurrence on farms regularly over a long period requires a lot of time and financial support may account for the lack of this type of study. Previous studies have investigated the effect of weather on animals by observing changes of the animals physiological systems. Some studies revealed the effect of weather on *Salmonella* under many different climatic conditions.

Climate has an effect on susceptible hosts and the agent capable of causing salmonellosis. The survivability of *S. senftenburg* in an aerosol chamber is dependent on the interaction of temperature and relative humidity, at low relative humidity (28%) increasing temperature within the range of 23-34 °C increases the tenacity of the organism, but the result is inverse at a high relative humidity (81%).²⁰ Another study found that salmonella viability at 72% relative humidity was approximately six times higher than the viability at 32%.⁹⁹ The effect of temperature on survival of *Salmonella* in the environment had been reported but the conclusion is not clear. Some studies showed that cooler temperatures were more beneficial for the organism but other studies showed opposite results. The number of *S. typhimurium* in cattle slurry declined more rapidly at warmer temperature (17 °C compared to 4 °C).⁴¹ *Salmonella typhimurium* reappeared in soil samples during winter after a long period (68 weeks) of absence from the environment.¹⁹ *Salmonella dublin* in slurry survived for 24 weeks on a pasture when the slurry was applied in October but for only 13 weeks when similar slurry was applied in March.²³ Paradoxically, a study showed that *S. dublin* survived for at least 73 days in feces on pasture in the winter and 119 days in the summer.¹⁰⁹ Survivability of *S. typhimurium* in cow urine at 6 °C was less than a day but at 21 °C it survived for 4 days.⁶⁷ The growth rate of salmonella organisms in raw beef increased as the temperature increased from 10 °C to 35 °C⁴⁷; in other words, replication or growth increased as the temperature approached the optimum growth temperature for *Salmonella* (35-37 °C).

It is generally known that severe climatic conditions cause stress in cattle. Continuous stress may result in immune deficiencies and increases the susceptibility to infections due to hyperglucocorticoidism.⁶ High environmental temperatures can increase levels of corticosteroids which results in diminishing host resistance mechanisms.³⁴ It also reduces the absorption of colostral immunoglobulin in newborn calves.³⁴ In winter,

the systemic resistance of animals may be lower because of the animals poor nutritional status at this time.¹⁰⁰

The change of weather conditions may be sufficient to affect the steady state between cattle, *Salmonella*, and the environment; consequently, it may result in a change in the frequency of disease in the population. This study was designed to investigate the potential effects of weather on the occurrence of salmonella positive samples from cattle in Virginia.

Review of Statistical Analysis

The objective of correlation analysis is to find the relationship between the two sets of measurements. The analysis determines if a set of explanatory variables is able to estimate a set of response variables. There are three steps of the statistical process given without order as follows:¹⁰¹

- 1) Finding the degree of relationship in a form of statistical measurement
- 2) Finding mathematical expressions that connect two sets of data together; usually called regression equations
- 3) Finding statistical tests of significance for the results of steps 1 and 2.

Correlation coefficients measure the degree of relationship between the two variables. Pearson's correlation coefficient may provide the best estimate of the population correlation coefficient if the data are normally distributed.¹² Spearman's Rank Correlation Coefficient (Rho or r_s) is recommended instead of Pearson's when the data do not appear to have normal distributions.¹² In some cases, it is possible to make a preliminary transformation of the data to normality; however, it can be difficult to interpret the results involving a correlation computed from transformed data.

Spearman s Rank Correlation Coefficient

Spearman's method converts every observation into rank and finds the correlation of ranks; as a result, some information is lost. The analysis is designed to measure the strength of the monotone relationship between two measurements and give the results in terms of the correlation coefficient. Spearman's Rho (r_s) is obtained as follows:⁸⁹

$$r_s = 1 - \frac{\sum_{i=1}^n d_i^2}{n(n^2 - 1)}$$

R_{xi} = rank of x_i value (x_i ranges from 1 to n , the smallest value is rank 1)

R_{yi} = rank of y_i value (rank is assigned the same way as R_x)

$d_i = R_{xi} - R_{yi}$, $i = 1, 2, \dots, n$

n is the number of observations

Spearman's Rho (r_s) measures the degree of association, but does not necessarily indicate the causality effect of variables. The value of r_s is always between +1 and -1. The value +1 means there is complete agreement between the two sets of ranks, and -1 means there is complete disagreement between the two sets of ranks. A negative correlation coefficient means that the two sets of data are inversely correlated and are directly correlated for a positive correlation coefficient.

Regression Analysis

The correlation coefficient measures the linear association between two variables, while a coefficient of a regression model measures the size of the change in the response variable, which can be predicted when a unit change is made in the explanatory variable. Regression analysis can be used to find an equation for the prediction of a response variable by knowing the values of one or more explanatory variables.

There is always a question in regression analysis as to how many and what variables should be included in the model when no model has previously been described. The variable selection procedure may be used to solve this problem. The procedures that have been widely used are: 1) All-possible-regression procedure 2) Backward elimination, 3) Forward selection, and 4) Stepwise procedure.⁴²

The All-possible-regression procedure is preferred over the other variable selection procedures because it is the only method that guarantees finding the best model. However, it has the disadvantage of potentially requiring more computation time. The procedure requires fitting all possible regression equations associated with the possible combinations of k independent variables. For k independent variables, the number of the models to be fitted is $2^k - 1$.⁴² Once all models have been fitted, the best model can be selected based on some criterion such as R^2 , MSE or C_p . The other three methods are alternate methods that do not guarantee optimum subsets of variables, but they are well known and widely used. The Forward selection and the Backward elimination have limitations because they cannot account for the effects of added or deleted variables on the contributions of other variables that are already in the model. A variable added to the model early in Forward selection can become unimportant after other variables are added, or variables previously dropped in Backward elimination can become important after the other variables are dropped from the model.⁶⁸ The Stepwise procedure combines both Forward and Backward procedures together and overcomes some of the major deficiencies in Forward and Backward methods due to the ability to recheck the importance of each regressor variable at each step of the procedure, so it is recommended the most.³¹

The optimum significant levels in the Stepwise variable selection methods are recommended to be 0.25 for the forward process and half that level for the backward process.⁶⁸ The variable selection should not be performed when the samples are as small as $n - p \leq 10$, n is the number of observations and p is the number of predictors.⁶⁸

Explanatory variables in regression analysis may be categorical factors; for example, breed, sex, and season. These categorical variables can be represented by indicator or dummy variables. The numbers 0 and 1 are usually used for the indicator variables. These numbers do not equal the values of variables, but identify category or class membership. The categorical coefficients can be visualized as a shift in the constant term, depending on which category the prediction is based.⁵⁶ Indicator variables may be considered whenever there are categorical factors affecting a relationship. When there are categorical variables in the models, the assumption of additivity is that the effects of the quantitative regressors remain the same across levels or classes of the categorical variables. The condition of nonadditivity can be modeled as a model containing interaction. One may model interaction terms and test hypotheses on these terms when

there is a concern about the additivity assumption. A multiple partial F-test or a t-test are usually used to detect the interaction. If the interaction terms are significant, the conclusion is as follows:⁵⁶

- i) The model without interaction terms is not appropriate.
- ii) The effect of quantitative variables is different for the different levels of categorical variables.
- iii) For model fitting, one should fit separate regression lines for the different levels of categorical variables.

When the data are collected continuously over a period of time, it is possible for a lack of independence of model residuals to occur. Some time-dependent factors such as population size or cyclic change may have influenced the results of the study. Potential consequences could include incorrect conclusions about the relationship among the study factors or misspecification of the model. Data sets that have correlated residuals over time are often called time series data.⁵⁶ The correlation of residuals is called autocorrelation (ρ) which has a maximum value of 1. Positive autocorrelation gives a smaller error variance; as a result, there is a higher chance to have a type I error. Negative autocorrelation inflates the error variance and reduces the power of the test.⁷⁵ To detect the correlation between adjacent observations, the Durbin-Watson test⁵⁶ is widely used. This test was designed for testing the autocorrelation of data that were taken over time. The null hypothesis versus the alternative hypothesis of this test is as follows:

$$H_0 : \rho = 0$$

$$H_1 : \rho > 0$$

If the null hypothesis is not rejected it means that residuals of time consecutive observations are uncorrelated. The test statistic of Durbin-Watson test is obtained from the following equation:

$$d = \frac{\sum_{i=2}^n (e_i - e_{i-1})^2}{\sum_{i=1}^n e_i^2}$$

e_i = residuals from the least squares analysis of the data

$e_i = y_i - \mu_{xi}$, $i = 1, 2, \dots, n$

The rules for hypothesis rejection are as follows:

- i) If $d < d_l$, reject H_0
- ii) If $d > d_u$, accept H_0
- iii) If $d_l \leq d \leq d_u$, decision is inconclusive

The value of d_l and d_u are available in a Durbin-Watson table which can be found in most regression analysis and econometrics textbooks.

When autocorrelation is not present, regular regression analysis can be utilized. The presence of autocorrelation can be handled by adjusting the regression model.⁵⁶ The new regression model includes the values of the dependent variable from the previous time period as an explanatory variable. In time series data, the observation values are assumed to be associated with the value of their previously adjacent observations which should be included in the model.

Multiple regression equations usually are interpreted as measuring the change in the dependent variables when an explanatory variable is increased by one unit and other explanatory variables are held constant. The interpretation may not be valid if there are strong linear relationships among explanatory variables. To change an explanatory variable while the other explanatory variables remain constant is almost impossible. This problem has been named “multicollinearity”. Multicollinearity may exist even though the model gives an acceptable residual distribution. It is generally believed that if multicollinearity occurs, one should consider variable deletion or an alternative to least squares estimation to solve the problem.⁵⁶

The regression model assumptions are³¹: 1) the model is correctly specified; 2) predictor variables are not random and are measured without error; 3) the residuals are independent from observation to observation, have zero means and constant variances; and 4) the model residuals follow a normal probability distribution.

The first assumption implies that all relevant predictor variables are appropriately included in the model and that they affect the response as linear terms. The second assumption states that the data are assumed to be random samples. Regression analysis is often used in the analysis of time series and when all members of a population are observed²⁵; this study is an example. The third and fourth assumptions are clearly stated and can be checked by the analysis of residuals.

Chapter 3.

Materials and Methods

Source of Data and Data Collection

Data were retrieved and entered into a spread sheet during the summers of 1995 and 1996. Records of four out of six regional diagnostic laboratories in the state of Virginia, USA were used. Records of the other two laboratories were not used because of low estimated number of positive samples. Regional diagnostic laboratories from which data were obtained were Harrisonburg, Lynchburg, Warrenton and Wytheville Regional Laboratories of the Virginia Department of Agriculture and Consumer Services, Animal Health Services (Figure 11). Completed records from the previous three to five years were available. The records of the Lynchburg and Warrenton laboratories were available for 1993 to 1995. The records in the Harrisonburg laboratory were available for the period between 1990 and 1994. The Wytheville laboratory had missing records from 1993 and October-December of 1992; therefore, only the available records from 1992 to 1995 were used. Laboratory submission records were thoroughly examined. Each individual record corresponded to one animal that had samples submitted to a laboratory regardless of the number of specimens collected from that animal. There were variations among data recording systems at the regional laboratories. For the Harrisonburg laboratory, samples submitted for salmonella cultures were samples specifically requested for salmonella cultures on submission forms. For the Wytheville and Warrenton laboratories, samples were fecal samples, samples from diarrheal cattle, or samples that had at least one gut specimen submitted for bacteriologic culture. At the Lynchburg laboratory, all cattle samples, except for milk samples, were cultured for salmonella bacteria. The salmonella culture protocols were slightly different among the laboratories (see Appendix 2). All salmonella isolates were submitted to the National Veterinary Services Laboratories (NVSL) at Ames, Iowa for serotyping.

Collected cattle data were composed of the monthly total count of bovine samples submitted for salmonella culture and the monthly number of bovine samples positive for salmonella culture (Figure 12). The information for the monthly total of bovine samples submitted for salmonella culture at the Warrenton laboratory was not retrievable. The date that samples were submitted were recorded in order to correspond the sample results to weather conditions. Serotypes of isolated salmonella bacteria were recorded as well.

The monthly percentage of positive samples was calculated by dividing the monthly number of positive bovine samples by the total monthly bovine samples cultured for salmonella then multiplying by 100.

Climatological data were obtained from the National Climatic Data Center (NCDC) monthly reports and the Virginia State Climatology Office at the University of Virginia. Three weather parameters measured monthly were used: total precipitation in inches, temperature in Fahrenheit, and average percent relative humidity. The temperature data included the average daily maximum, average daily minimum, average daily mean, highest, and lowest temperature. The daily mean temperature was calculated by averaging the daily maximum and daily minimum temperature. Data on the average

percent relative humidity were only available from the University of Virginia records for two studied regions. Each regional laboratory was matched with the closest weather station in order to detect a correlation of salmonella culture results with the temperature and precipitation. The Harrisonburg, Lynchburg, Warrenton, and Wytheville laboratories were matched with Dale Enterprise, Lynchburg WSO Airport, Warrenton 3 SE and Wytheville 1 S weather stations, respectively. Relative humidity data were obtained from two locations, Roanoke and Lynchburg. The Roanoke station was matched with the Harrisonburg and Wytheville laboratories; while the Lynchburg station was matched with the Lynchburg and Warrenton laboratories.

For descriptive statistics, the monthly average for each variable was calculated by averaging all observations during the period that data were collected. The outcomes were the average values for each calendar month. Data were put into a spreadsheet for further statistical analysis.

Statistical Analysis

Data from each of the four laboratories were separately analyzed by using SAS[®] statistical software.⁷⁶

Descriptive Analysis

The descriptive statistics were performed for the number and the percentage of bovine samples that had positive salmonella cultures. The total number of bovine samples submitted for cultures at the Warrenton laboratory were not available, so some of the descriptive statistics for this laboratory were not performed. Serotypes of *Salmonella* that were isolated each year and their percentage of the total number of isolates were recorded for each laboratory. The percentage of serotypes isolated from the number of cultured samples was calculated as well. The arithmetic means of the number and percentage of positive samples were summarized by calendar month for each laboratory.

Monthly bovine sample data were stratified into four seasonal categories-- winter, spring, summer and fall. December, January and February were in the winter category. The next three monthly series were in the spring, summer, and fall, respectively. The average values for the monthly number and percentage of positive samples were calculated by season and laboratory.

Correlation Analysis

Before the correlation coefficients were calculated, the data for each variable were tested for normality using the UNIVARIATE procedure in SAS[®] statistical software. The Pearson's correlation coefficient was used when the data appeared to be normal. If the results showed that many of the variables were not normally distributed, the Spearman Rank Test was used for the correlation analysis. The Spearman's Rank Correlation Coefficients and p-values were calculated using the CORR procedure in the SAS[®] software. To determine the association of the previous month's weather and the previous two months' weather on the development of salmonellosis, the correlation coefficients of the positive samples and the previous one and two months' weather values were also examined. Five measurements of the temperature were considered for correlation analysis: average maximum, average minimum, average, highest monthly temperature and lowest monthly temperature. Preliminary analysis using Pearson's correlation coefficient showed very high correlation among these measurements ($r > 0.9$, $p < 0.01$); therefore, only the monthly average temperature was selected. The monthly average temperature was selected as it is reported by the majority of weather facilities. Correlation coefficients were calculated between each weather parameter: the average temperature, total precipitation, and average relative humidity and salmonella culture results measured as the monthly number of positives and the monthly percentage positives.

Cattle salmonellosis outbreaks occurred in the summer of 1994 in northwestern Virginia where the Harrisonburg laboratory was located. The causes of the outbreaks were not known. Samples from these outbreaks may result in the association of the

disease and the weather in that area; therefore, the correlation coefficients were calculated with and without the 1994 data. Additional analysis was performed in order to eliminate the effect of 1994 outbreaks on the correlation coefficient between the response variables and the weather parameters.

Regression Analysis

The REG procedure in SAS[®] statistical software was used for the linear regression analysis. The response variables used in the regression model were the monthly number and monthly percentage of salmonella positive cultures. In the first step, the explanatory variables were selected by using the Stepwise Procedure to select variables from the average temperature, precipitation, and the relative humidity of the same and the previous one and two months at $\alpha=0.25$ for the forward selection step and $\alpha=0.10$ for the backward elimination step. The model obtained from the Stepwise Procedure had selected weather parameters as regressor variables. The season of the year was considered as a categorical variable with four classes; winter, spring, summer, and fall. These seasonal classes were modeled with three indicator variables containing values of 0 and 1. The indicator variables accounted for the different effects of seasons in which the positive samples were collected. The winter category, which contained the lowest number and percentage of positive samples at the Harrisonburg laboratory, was used as a baseline. The regression model contained z_1 - z_3 as indicator variables. The regression model, provided that the Stepwise Procedure result had only one weather parameter, was given by:

$$(1) \quad y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 z_{1i} + \beta_3 z_{2i} + \beta_4 z_{3i} + \epsilon_i \quad , i = 1, 2, 3, \dots, n$$

where as,

β_0	= a constant value
β_1 - β_4	= coefficients of the regressor variables
x_1	= weather parameter obtained from Stepwise Procedure
z_1	= 1 if the ith. respondent is in the spring season 0 otherwise
z_2	= 1 if the ith. respondent is in the summer season 0 otherwise
z_3	= 1 if the ith. respondent is in the fall season 0 otherwise
ϵ	= residual
n	= the number of observations

The interaction terms were introduced into the model to test the additivity effect of weather parameters and seasons. When the additivity effect held, the effect of weather parameter was the same across all seasons. To test this, the followed model was used:

$$(2) \quad y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 z_{1i} + \beta_3 z_{2i} + \beta_4 z_{3i} + \beta_{1z_1} x_{1i} z_{1i} + \beta_{1z_2} x_{1i} z_{2i} + \beta_{1z_3} x_{1i} z_{3i} + \epsilon_i$$

, $i = 1, 2, 3, \dots, n$

Then the following null hypothesis was tested:

$$H_0: \beta_{1z_1} = \beta_{1z_2} = \beta_{1z_3} = 0$$

According to this null hypothesis, the following statement must be true:

$$R(\beta_1, \beta_2, \beta_3, \beta_4, \beta_{1z_1}, \beta_{1z_2}, \beta_{1z_3} / \beta_0) - R(\beta_1, \beta_2, \beta_3, \beta_4 / \beta_0) =$$

$$R(\beta_{1z_1}, \beta_{1z_2}, \beta_{1z_3} / \beta_0, \beta_1, \beta_2, \beta_3, \beta_4)$$

These regression sums of squares are in ANOVA tables obtained from regression analyses using the model 1 and 2. An F-test for testing the null hypothesis was performed as follows:

$$F = \frac{R(\beta_{1z_1}, \beta_{1z_2}, \beta_{1z_3} / \beta_0, \beta_1, \beta_2, \beta_3, \beta_4) / 3}{MSE_{full}}$$

- notes: i) degree of freedom for the F is 3, the difference in parameter numbers of full and reduced models for the numerator
 ii) degree of freedom for the denominator is the degree of freedom of the mean square error in the full model

Multicollinearity was detected by regressing the weather parameter (x_i) against other explanatory variables in the same model (x_j , $j \neq i$). The relationships among explanatory variables were explained by the coefficients of determinations which determined the removal of indicator variables. Afterward, the season indicator variables were deleted from the model of the Harrisonburg and Wytheville laboratories due to strong relationships with the selected weather parameters.

The Durbin-Watson Test was performed as the next step to detect any possible autocorrelation among observations. If autocorrelation existed at the level of significance (0.05), then the value of positive samples from the previous month were added to the regression model. Transformation of the response variables was performed in order to produce satisfactory residual distributions. Some simple transformations, for example, natural log, square root, inverse sine, and reciprocal values, were tried and the selected transformation was based on the plot of residual against the prediction values of dependent variables. The final model, provided that the interaction terms were not significant, was as follows:

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 y_{i-1} + \beta_3 z_{1i} + \beta_4 z_{2i} + \beta_5 z_{3i} + \epsilon_i \quad , i = 1, 2, 3, \dots, n$$

where as,

β_0	= a constant value
β_1 - β_5	= coefficients of the predictor variables
x_1	= weather parameter obtained from Stepwise Procedure
z_1	= 1 if the ith. respondent is in the spring season 0 otherwise
z_2	= 1 if the ith. respondent is in the summer season 0 otherwise
z_3	= 1 if the ith. respondent is in the fall season 0 otherwise
ϵ	= residual
n	= the number of observations

notes: y_{i-1} was added in the model when the Durbin-Watson test showed significant autocorrelation. z_1 - z_3 were added in the model only if the multicollinearity was not a problem.

The normality and constant variance of the residuals were checked by using the UNIVARIATE procedure in SAS[®], and the plots between the residuals and the predicted values of the response variables.

Chapter 4.

Results

Descriptive Analysis

The Harrisonburg laboratory had the highest annual number and percentage of positive samples, followed by the Wytheville and Lynchburg laboratories (Table 1). The number of positive samples at the Harrisonburg laboratory in 1994 was approximately three times higher than the positive numbers of the other years because of the unusually high number of outbreaks in the summer of 1994. The serotype isolated most often from bovine samples submitted for salmonella culture was *S. typhimurium*, 75.2% of positive samples (Table 2).

The Harrisonburg laboratory, which had the highest frequency of reported isolations, had higher medians for the number and for the percentage of salmonellosis in summer and fall than in winter and spring (Table 3, 4). The pattern of the salmonellosis occurrence for different seasons was inconsistent among the laboratories (Figure 2, 3).

The highest value for the average monthly number of positive samples at Harrisonburg was in September (4.4); January (2.0) at the Lynchburg laboratory; November (1.7) at the Warrenton laboratory; and February (3.7) at the Wytheville laboratory (Table 5). The average monthly percentage of positive samples was highest in September (26.5%), January (15.3%), and April (38.9%) for the Harrisonburg, Lynchburg and Wytheville laboratories, respectively (Table 6).

Table 1. THE PERCENTAGE OF SAMPLES POSITIVE FOR SALMONELLA CULTURE AND THE SEROTYPES OF ISOLATED SALMONELLA

Laboratory	Year	Positive Percentage	Serotypes ^a	Serotypes Percentage
Harrisonburg	1990	11.1% (22/198)	<i>S. typhimurium</i>	36.4% (8/22)
			<i>S. hadar</i>	22.7% (5/22)
	1991	11.1% (20/180)	<i>S. typhimurium</i>	70.0% (14/20)
			<i>S. heidelberg</i>	20.0% (4/20)
	1992	10.6% (25/236)	<i>S. typhimurium</i> <i>S. heidelberg</i>	72.0% (18/25) 12.0% (3/25)
Lynchburg	1993	20.8% (22/106)	<i>S. typhimurium</i>	45.5% (10/22)
			<i>S. kentucky</i>	13.6% (3/22)
	1994	30.4% (62/204)	<i>S. typhimurium</i>	87.0% (54/62)
			<i>S. anatum</i>	4.8% (3/62)
	1995	5.2% (10/193)	<i>S. typhimurium</i> others	60%(6/10) 40%(4/10)
Warrenton	1994	3.1% (4/128)	<i>S. typhimurium</i>	100%(4/4)
	1995	7.4% (7/94)	<i>S. typhimurium</i> <i>S. thompson</i>	85.7%(6/7) 14.3%(1/7)
	1996	-	-	-
Wytheville	1993	-	<i>S. typhimurium</i>	100%(3/3)
	1994	-	<i>S. typhimurium</i> others	84.6%(11/13) 15.4%(2/13)
	1995	-	<i>S. typhimurium</i>	100%(2/2)
Wytheville	1994	6.7% (7/104)	<i>S. typhimurium</i>	85.7%(6/7)
			<i>S. berta</i>	14.3%(1/7)
	1995	10.8% (14/139)	<i>S. typhimurium</i>	100%(14/14)

^aOnly the first and second most frequently isolated serotypes are shown in the table.

Table 2. THE SEROTYPES OF ISOLATED SALMONELLA ORGANISMS FROM STUDIED LABORATORIES AND THEIR PERCENTAGES

serotypes	percentage	serotypes	percentage
<i>S. typhimurium</i>	75.23 %	<i>S. ohio</i>	0.90 %
<i>S. heidelberg</i>	4.05 %	<i>S. berta</i>	0.45 %
<i>S. kentucky</i>	2.70 %	<i>S. blockley</i>	0.45 %
<i>S. hadar</i>	2.25 %	<i>S. dublin</i>	0.45 %
<i>S. muenster</i>	2.25 %	<i>S. litchfield</i>	0.45 %
<i>S. anatum</i>	1.35 %	<i>S. london</i>	0.45 %
<i>S. arizona</i>	1.35 %	<i>S. mbandaka</i>	0.45 %
<i>S. enteritidis</i>	1.35 %	<i>S. oranienberg</i>	0.45 %
<i>S. thompson</i>	1.35 %	<i>S. schwarzengrund</i>	0.45 %
<i>S. infantis</i>	0.90 %	unable to identify	2.25 %
<i>S. muenchen</i>	0.90 %		

Table 3. MEDIAN VALUES OF THE MONTHLY NUMBER OF SAMPLES POSITIVE FOR SALMONELLA CULTURE BY SEASON

Laboratory	Season			
	winter	spring	summer	fall
Harrisonburg	1	1	3	3
Lynchburg	1	0	1	0
Warrenton	0	0	0	1
Wytheville	1	1	0	1

Table 4. MEDIAN VALUES OF THE MONTHLY PERCENTAGE OF SAMPLES POSITIVE FOR SALMONELLA CULTURE BY SEASON

Laboratory	Season			
	winter	spring	summer	fall
Harrisonburg	7.1	11.1	20.0	18.8
Lynchburg	7.7	0	5.9	0
Wytheville	8.1	12.5	0	7.7

Note: No percentage is shown for the Warrenton laboratory due to unretrievable number of the total bovine samples submitted for salmonella culture

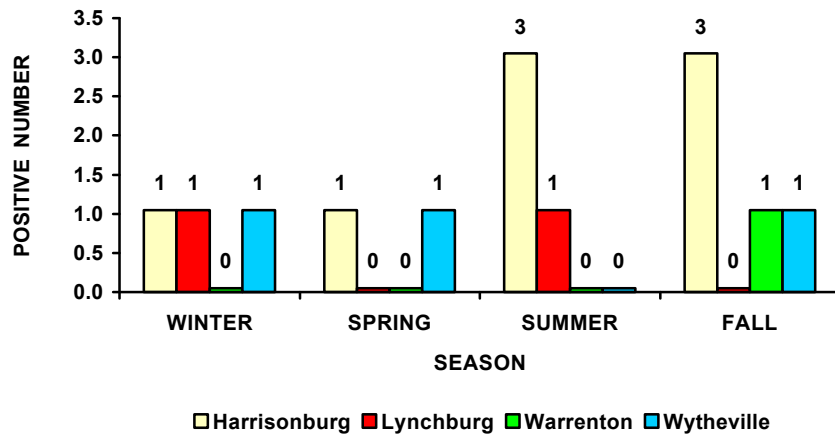
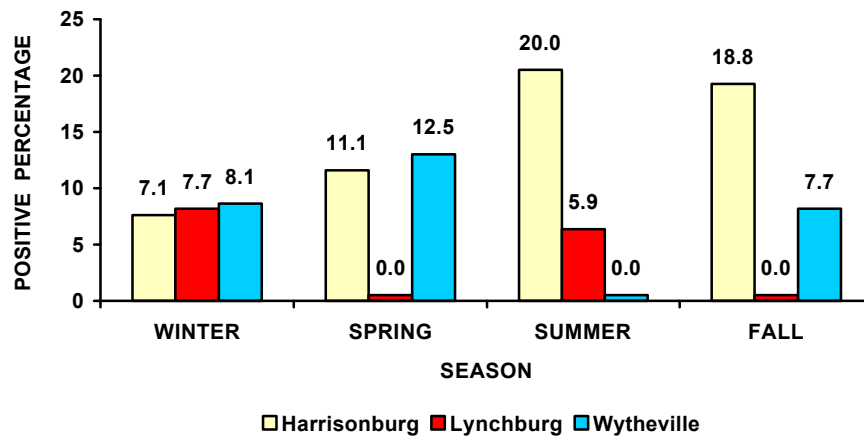


Figure 2. THE MEDIAN VALUES OF THE MONTHLY NUMBER OF POSITIVE SAMPLES FOR SALMONELLA CULTURE BY SEASON



Note: No percentage is shown for the Warrenton laboratory due to unretrievable number of the total bovine samples submitted for salmonella culture

Figure 3. THE MEDIAN VALUES OF THE MONTHLY PERCENTAGE OF POSITIVE SAMPLES FOR SALMONELLA CULTURE BY SEASON

Table 5. THE NUMBER OF SAMPLES POSITIVE FOR SALMONELLA
CULTURE AVERAGED BY MONTH AND LABORATORY

Month	Laboratory			
	Harrisonburg ^a	Lynchburg ^b	Warrenton ^b	Wytheville ^c
January	0.8	2.0	0.7	0.3
February	1.4	1.0	0.0	3.7
March	1.8	0.0	1.3	0.7
April	2.2	0.0	0.0	2.3
May	2.6	0.0	0.3	0.7
June	2.4	0.7	0.3	0.0
July	3.2	1.0	0.3	0.3
August	4.2	0.7	0.3	0.0
September	4.4	0.0	0.3	0.7
October	3.2	0.0	0.7	0.5
November	2.4	0.7	1.7	1.5
December	1.6	1.0	0.0	1.0

^aData were averaged during 1990-1994

^bData were averaged during 1993-1995

^cData were averaged during January-September 1992 and 1994-1995

Table 6. THE PERCENTAGE OF SAMPLES POSITIVE FOR SALMONELLA CULTURE AVERAGED BY MONTH AND LABORATORY

Month	Laboratory		
	Harrisonburg ^a	Lynchburg ^b	Wytheville ^c
January	8.4	15.3	3.3
February	9.8	5.9	17.7
March	10.1	0.0	12.6
April	13.4	0.0	38.9
May	22.4	0.0	8.9
June	13.8	13.3	0.0
July	22.3	8.6	3.7
August	24.2	10.4	0.0
September	26.5	0.0	3.9
October	22.0	0.0	3.8
November	11.7	5.6	22.5
December	8.2	9.1	33.3

^aData were averaged during 1990-1994

^bData were averaged during 1993-1995

^cData were averaged during January-September 1992 and 1994-1995

Descriptive Statistics of the Weather Variables

The mean of temperature by month, which was obtained by averaging the monthly temperature during the period that the bovine samples were collected, had the same trend with the highest temperature occurring in July. The Wytheville 1 S station, which was matched with the Wytheville laboratory, had a slightly lower average temperature than the other weather stations from April to December (Figure 4). The values of relative humidity which were averaged by month for three different laboratories were very similar and remained over 70% for almost the whole year (Figure 5). The relative humidity data for the Harrisonburg and Wytheville laboratories were from the same weather station but they were averaged during different time periods depending on the year that cattle data were retrieved. The monthly average precipitation did not appear to have the same seasonal pattern for every weather station (Figure 6).

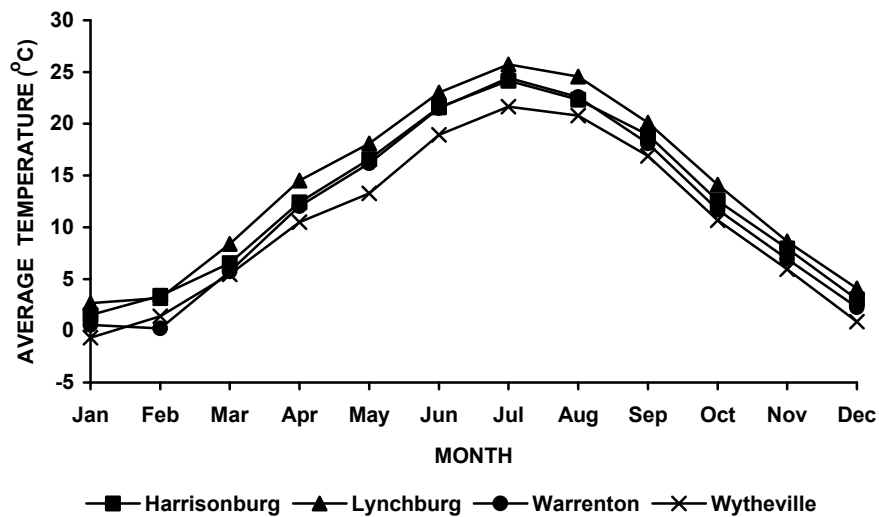


Figure 4. THE TEMPERATURE AVERAGED BY MONTH FOR EACH LABORATORY

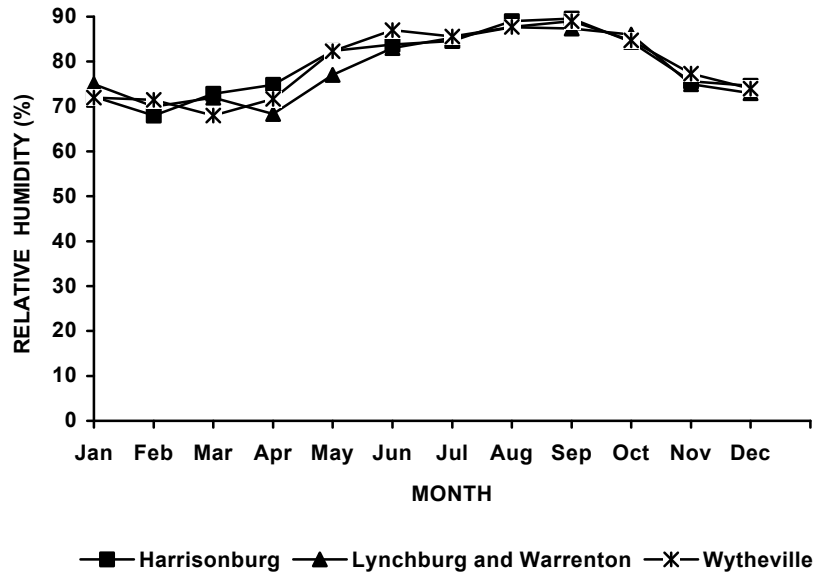


Figure 5. THE RELATIVE HUMIDITY AVERAGED BY MONTH FOR EACH LABORATORY

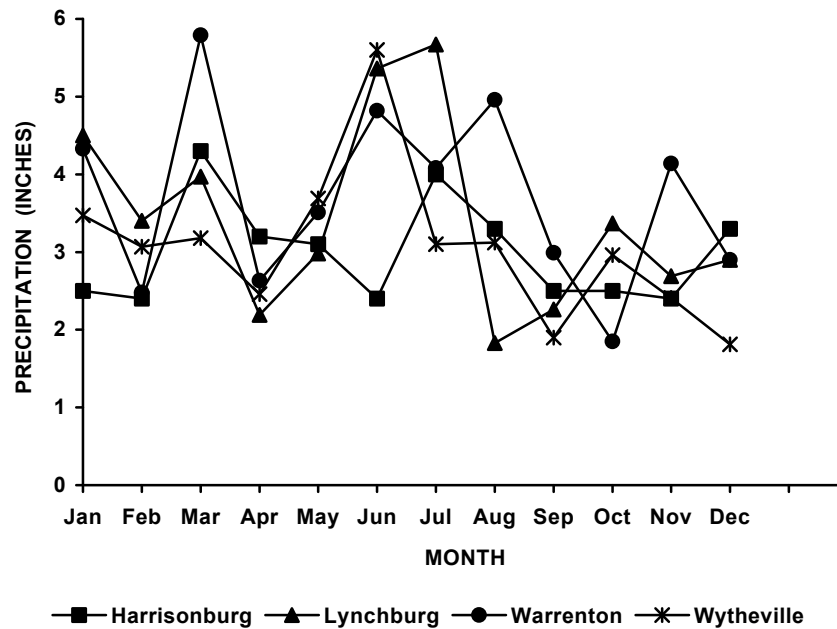


Figure 6. THE TOTAL PRECIPITATION AVERAGED BY MONTH FOR EACH LABORATORY

Distribution of the Data

Data sets of the weather variables were normally distributed, but none of the monthly number of bovine samples positive for salmonella culture had a normal distribution. The monthly number and percentage of positive samples are summarized in Table 7.

Table 7. THE DESCRIPTIVE STATISTICS OF THE MONTHLY NUMBER AND PERCENTAGE OF THE POSITIVE SAMPLES: THE MAXIMUM, MEDIAN AND MINIMUM VALUES

Laboratory	Monthly Number			Monthly Percentage		
	max.	med.	min.	max.	med.	min.
Harrisonburg	11	2	0	57.9	12.9	0
Lynchburg	3	0	0	40.0	0	0
Warrenton	4	0	0	-	-	-
Wytheville	6	0	0	100	0	0

Correlation Analysis of Weather Variables

There were highly significant correlations ($r > 0.9$, $p < 0.001$) among the monthly average temperature and other measures of temperature: the average maximum, the average minimum, the highest and the lowest temperatures (Table 8). These high correlations were detected from data of all four weather stations used in this study. The average temperature values were also positively correlated to the values of the relative humidity ($p < 0.001$) while the total precipitation values were not significantly correlated ($p > 0.05$) to any other weather variables (Table 9).

Table 8. PEARSON CORRELATION COEFFICIENTS BETWEEN THE MONTHLY AVERAGE TEMPERATURE AND THE MONTHLY AVERAGE MAXIMUM, AVERAGE MINIMUM, HIGHEST AND LOWEST TEMPERATURE, TOTAL PRECIPITATION, AND AVERAGE RELATIVE HUMIDITY

Station	Max.	Min.	Highest	Lowest	Precip.	RH.
Dale Enterprise ^b	0.996	0.994	0.961	0.963	0.036 ^a	0.753
Lynchburg WSO Airport ^c	0.997	0.997	0.961	0.974	0.017 ^a	0.654
Warrenton 3 SE ^c	0.992	0.991	0.922	0.979	0.010 ^a	0.609
Wytheville 1 S ^b	0.993	0.992	0.953	0.978	0.171 ^a	0.775

All p-values < 0.001, except for ^ap-values > 0.05

^bUsing the relative humidity data from Roanoke Station

^cUsing the relative humidity data from Lynchburg Station

Table 9. PEARSON CORRELATION COEFFICIENTS BETWEEN THE MONTHLY TOTAL PRECIPITATION AND THE MONTHLY AVERAGE RELATIVE HUMIDITY

Station	r
Dale Enterprise ^a	0.078
Lynchburg WSO Airport ^b	0.281
Warrenton 3 SE ^b	0.295
Wytheville 1 S ^a	0.211

All p-values > 0.05

^aUsing the relative humidity data from Roanoke Station

^bUsing the relative humidity data from Lynchburg Station

Correlation of the Monthly Number of Samples Positive for Salmonella Culture and the Monthly Average Temperature

At the Harrisonburg and Wytheville laboratories, significant correlations were detected between the number of positive samples and the temperature (Table 10). For the Harrisonburg laboratory, the highest correlation coefficient was found when using the average temperature of the previous month ($r_s=0.30$, $p=0.02$). The monthly positive number at the Wytheville laboratory was inversely correlated to the average temperature, and the highest correlation was obtained by using the average temperature of the same month ($r_s= -0.38$, $p=0.03$). Association between the monthly positive number and the monthly temperature of Harrisonburg's data is shown in Figure 7.

Table 10. SPEARMAN CORRELATION COEFFICIENTS BETWEEN THE MONTHLY NUMBER OF POSITIVE SAMPLES AND THE MONTHLY AVERAGE TEMPERATURE AND THEIR P-VALUES

Laboratory	Temperature			n
	avg. temp. ^a	1m. pre. temp. ^b	2m. pre. temp. ^c	
Harrisonburg	0.25	0.30	0.23	60
	0.06	0.02	0.08	
Lynchburg	-0.17	-0.02	0.05	36
	0.33	0.89	0.78	
Warrenton	-0.08	0.05	0.12	35
	0.66	0.77	0.48	
Wytheville	-0.38	-0.33	-0.21	33
	0.03	0.06	0.23	

^aThe average temperature of the same month as the monthly positive number

^bThe average temperature of the first preceding month

^cThe average temperature of the second preceding month

upper values: correlation coefficients

lower values: p-values

Correlation of the Monthly Percentage of Samples Positive for Salmonella Culture and the Monthly Average Temperature

Significant relationships were found from the Harrisonburg and Wytheville laboratories' data; however, the correlations had opposite meanings (Table 11). The correlations of Harrisonburg monthly percentage were positively correlated to the average temperatures while Wytheville's were negatively correlated. Similar to the correlation of the positive number, Harrisonburg had the highest correlation with the average temperature of the previous month ($r_s=0.34$, $p=0.01$), but Wytheville had the highest correlation with the average temperature of the same month ($r_s= -0.38$, $p=0.03$). Association between the monthly positive percentage and the monthly temperature of Harrisonburg's data is shown in Figure 8.

Table 11. SPEARMEN CORRELATION COEFFICIENTS BETWEEN THE MONTHLY PERCENTAGE OF POSITIVE SAMPLES AND THE MONTHLY AVERAGE TEMPERATURE AND THEIR P-VALUES

Laboratory	Temperature			n
	avg. temp. ^a	1m. pre. temp. ^b	2m. pre. temp. ^c	
Harrisonburg	0.29	0.34	0.27	60
	0.03	0.01	0.04	
Lynchburg	-0.10	0.04	0.09	36
	0.54	0.83	0.62	
Wytheville	-0.38	-0.32	-0.24	33
	0.03	0.07	0.18	

^aThe average temperature of the same month as the monthly positive percentage

^bThe average temperature of the first preceding month

^cThe average temperature of the second preceding month

upper values: correlation coefficients

lower values: p-values

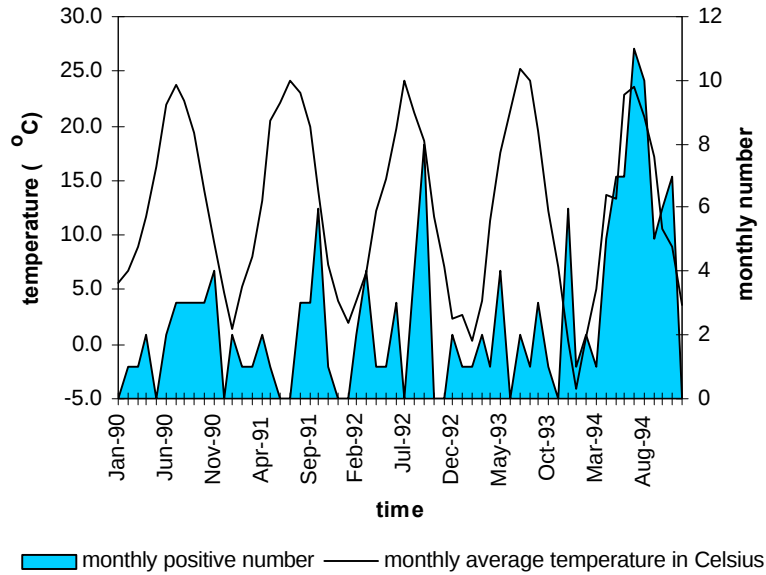


Figure 7. ASSOCIATION OF HARRISONBURG'S MONTHLY NUMBER OF SAMPLES POSITIVE FOR SALMONELLA CULTURE WITH THE MONTHLY AVERAGE TEMPERATURE

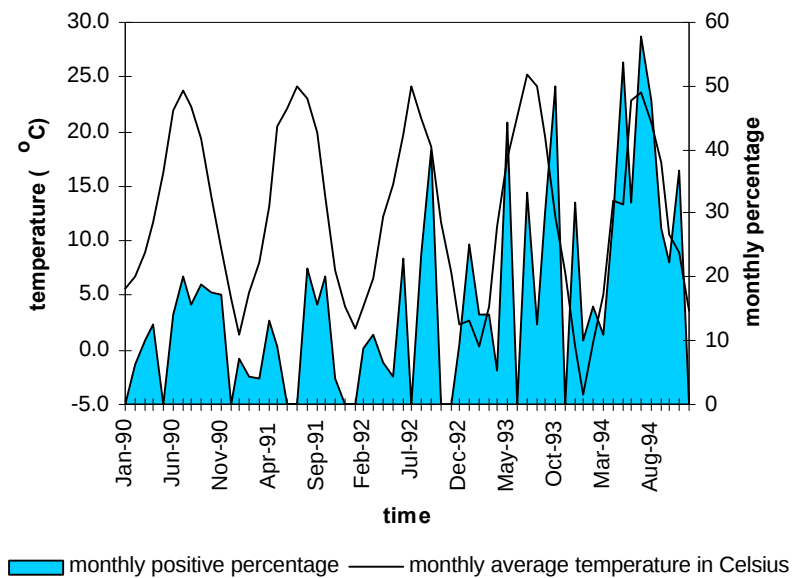


Figure 8. ASSOCIATION OF HARRISONBURG'S MONTHLY PERCENTAGE OF SAMPLES POSITIVE FOR SALMONELLA CULTURE WITH THE MONTHLY AVERAGE TEMPERATURE

Correlation of the Monthly Number of Samples Positive for Salmonella Culture and the Total Monthly Precipitation

Correlations between the monthly number of positive samples and the monthly precipitation were not statistically significant ($p > 0.05$). Differences among the trends of the correlations existed. Harrisonburg and Wytheville cattle data tended to be inversely related to the precipitation of the same month while that of Lynchburg and Warrenton tended to be positively related (Table 12).

Table 12. SPEARMAN CORRELATION COEFFICIENTS BETWEEN THE MONTHLY NUMBER OF POSITIVE SAMPLES AND THE MONTHLY PRECIPITATION AND THEIR P-VALUES

Laboratory	Precipitation			n
	precip. ^a	1m. pre. precip. ^b	2m. pre. precip. ^c	
Harrisonburg	-0.15	0.13	0.13	60
	0.26	0.33	0.32	
Lynchburg	0.17	0.17	0.13	36
	0.32	0.32	0.43	
Warrenton	0.24	-0.17	0.19	36
	0.16	0.32	0.27	
Wytheville	-0.13	-0.20	-0.30	33
	0.47	0.27	0.09	

^aThe total precipitation of the same month as the monthly positive number

^bThe total precipitation of the first preceding month

^cThe total precipitation of the second preceding month

upper values: correlation coefficients

lower values: p-values

Correlation of the Monthly Percentage of Samples Positive for Salmonella Culture and the Monthly Total Precipitation

Correlation of the monthly percentage of samples positive for salmonella culture and the monthly total precipitation were not significant ($p > 0.05$) (Table 13). The Wytheville correlations of the percentage positives with the precipitation and the previous months values were negative.

Table 13. SPEARMAN CORRELATION COEFFICIENTS BETWEEN THE MONTHLY PERCENTAGE OF POSITIVE SAMPLES AND THE MONTHLY PRECIPITATION AND THEIR P-VALUES

Laboratory	Precipitation			n
	precip. ^a	1m. pre. precip. ^b	2m. pre. precip. ^c	
Harrisonburg	-0.14	0.20	0.19	60
	0.29	0.12	0.14	
Lynchburg	0.14	0.17	0.13	36
	0.41	0.32	0.45	
Wytheville	-0.15	-0.14	-0.29	33
	0.40	0.44	0.10	

^aThe total precipitation of the same month as the monthly positive percentage

^bThe total precipitation of the first preceding month

^cThe total precipitation of the second preceding month

upper values: correlation coefficients

lower values: p-values

Correlation of the Monthly Number of Samples Positive for Salmonella Culture and the Monthly Average Relative Humidity

Harrisonburg had significant correlations with the relative humidity of the same month and the relative humidity of the previous month ($p \leq 0.02$). The relative humidity of the same month gave the highest correlation ($r_s=0.37$) compared to the humidity of the previous month ($r_s=0.30$). Wytheville data also had significant correlation and the highest correlation was the correlation with the relative humidity of the same month; however, the correlation was negative ($r_s= -0.39$, $p=0.03$). The correlation coefficients between the monthly number of salmonella positive samples and the monthly average relative humidity are shown in Table 14. The relationship between the monthly positive number and the monthly relative humidity is shown in Figure 9.

Table 14. SPEARMAN CORRELATION COEFFICIENTS BETWEEN THE MONTHLY NUMBER OF POSITIVE SAMPLES AND THE MONTHLY AVERAGE RELATIVE HUMIDITY AND THEIR P-VALUES

Laboratory	Relative Humidity			n
	humidity ^a	1m. pre. humid. ^b	2m. pre. humid. ^c	
Harrisonburg	0.37	0.30	0.08	58
	0.004	0.02	0.57	
Lynchburg	-0.04	0.17	0.13	36
	0.82	0.32	0.46	
Warrenton	-0.06	0.32	0.22	36
	0.70	0.05	0.19	
Wytheville	-0.39	-0.27	-0.07	32
	0.03	0.14	0.72	

^aThe average relative humidity of the same month as the monthly positive number

^bThe average relative humidity of the first preceding month

^cThe average relative humidity of the second preceding month

upper values: correlation coefficients

lower values: p-values

Correlation of the Monthly Percentage of Samples Positive for Salmonella Culture and the Monthly Average Relative Humidity

Similar to the correlations of the monthly positive number, Harrisonburg and Wytheville had significant correlations with the relative humidity ($p \leq 0.02$) and the correlations were highest when using the relative humidity of the same month (Table 15). The tendency of the relationship between Wytheville's data and the relative humidity was inverse ($r_s = -0.41$) but Harrisonburg's was positive ($r_s = 0.39$). The relationship between the Harrisonburg's percentage of positive samples and the relative humidity is displayed in Figure 10.

Table 15. SPEARMAN CORRELATION COEFFICIENTS BETWEEN THE MONTHLY PERCENTAGE OF POSITIVE SAMPLES AND THE MONTHLY AVERAGE RELATIVE HUMIDITY AND THEIR P-VALUES

Laboratory	Relative Humidity			n
	humidity ^a	1m. pre. humid. ^b	2m. pre. humid. ^c	
Harrisonburg	0.39 0.002	0.31 0.02	0.11 0.42	58
Lynchburg	0.01 0.94	0.21 0.23	0.10 0.55	36
Wytheville	-0.41 0.02	-0.33 0.07	-0.12 0.51	32

^aThe average relative humidity of the same month as the monthly positive percentage

^bThe average relative humidity of the first preceding month

^cThe average relative humidity of the second preceding month

upper values: correlation coefficients

lower values: p-values

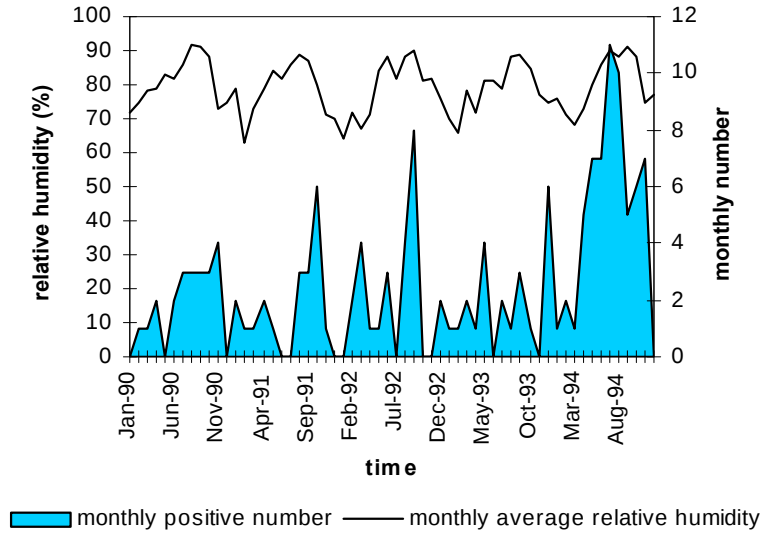


Figure 9. ASSOCIATION OF HARRISONBURG'S MONTHLY NUMBER OF SAMPLES POSITIVE FOR SALMONELLA CULTURE WITH THE MONTHLY AVERAGE RELATIVE HUMIDITY

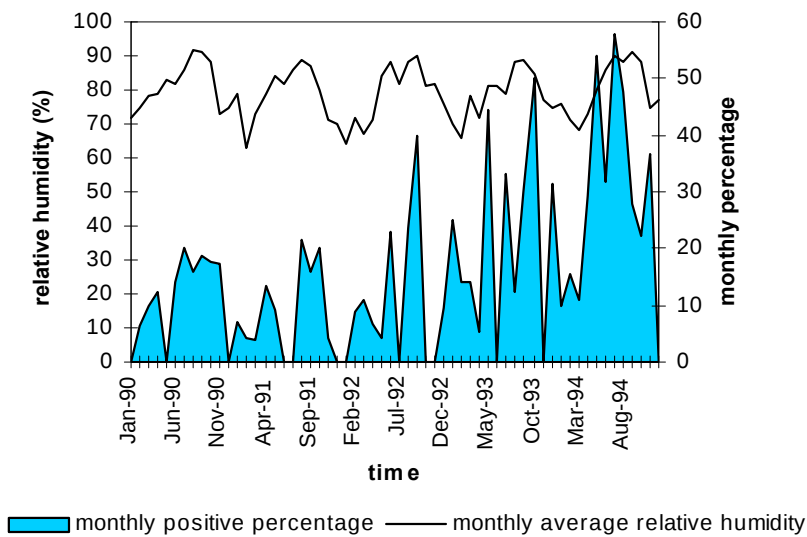


Figure 10. ASSOCIATION OF HARRISONBURG'S MONTHLY PERCENTAGE OF SAMPLES POSITIVE FOR SALMONELLA CULTURE WITH THE MONTHLY AVERAGE RELATIVE HUMIDITY

Correlations of Salmonella Positive Samples and the Weather at the Harrisonburg Laboratory with and without the Data of 1994

All of the correlation coefficients of the average temperature and the average relative humidity with the Harrisonburg's monthly number of positive samples were reduced when the 1994's data were excluded (Table 16). Nevertheless, the direction of the correlation coefficients remained positive. Without the data of 1994, none of the correlation coefficients were statistically significant at the 0.05 level of significance, except for the correlation with the relative humidity of the same month.

There was a reduction in the correlation coefficients between the monthly percentage of positive samples and number of monthly positive samples and the monthly average temperature when the data of 1994 were removed (Table 17). The correlation coefficients still had the same direction after the removal of the 1994 data.

Table 16. SPEARMAN CORRELATION COEFFICIENTS OF THE MONTHLY NUMBER OF POSITIVE SAMPLES AND THE WEATHER AT HARRISONBURG LABORATORY WITH AND WITHOUT THE DATA OF 1994

Weather parameter	1994 included	Without 1994
Avg.temp. ^a of the same month	0.25 0.06	0.14 0.34
Avg. temp. ^a one month before	0.30 0.02	0.26 0.08
Avg. temp. ^a two months before	0.23 0.08	0.21 0.14
Precip. ^b of the same month	-0.15 0.26	-0.18 0.22
Precip. ^b one month before	0.13 0.33	0.19 0.19
Precip. ^b two months before	0.13 0.32	0.09 0.56
RH. ^c of the same month	0.37 0.004	0.31 0.03
RH. ^c one month before	0.30 0.02	0.23 0.12
RH. ^c two months before	0.08 0.57	0.08 0.60

^aMonthly average temperature

^bMonthly total precipitation

^cMonthly average percent relative humidity

upper values: correlation coefficients

lower values: p-values

Table 17. SPEARMAN CORRELATION COEFFICIENTS OF THE MONTHLY PERCENTAGE OF POSITIVE SAMPLES AND THE WEATHER AT HARRISONBURG LABORATORY WITH AND WITHOUT THE DATA OF 1994

Weather parameter	1994 included	Without 1994
Avg.temp. ^a of the same month	0.29 0.03	0.21 0.15
Avg. temp. ^a one month before	0.34 0.01	0.32 0.03
Avg. temp. ^a two months before	0.27 0.04	0.29 0.05
Precip. ^b of the same month	-0.14 0.29	-0.20 0.17
Precip. ^b one month before	0.20 0.12	0.27 0.06
Precip. ^b two months before	0.19 0.14	0.18 0.22
RH. ^c of the same month	0.39 0.002	0.37 0.01
RH. ^c one month before	0.31 0.02	0.28 0.06
RH. ^c two months before	0.11 0.42	0.14 0.37

^aMonthly average temperature

^bMonthly total precipitation

^cMonthly average percent relative humidity

upper values: correlation coefficients

lower values: p-values

Regression Analysis for the Monthly Number and Percentage of Samples Positive for Salmonella Culture

The final regression models are composed of regressor variables from the Stepwise Procedure and, in the case of having autocorrelation, the lagging values of the positive number or percentage as another regressor variable was included. For the Warrenton laboratory, the model also has the indicator variables which accounted for the difference of seasons in which samples were collected. The indicator variables were deleted from the models of the Harrisonburg and Wytheville laboratories due to multicollinearity between the weather parameters and the seasons. The monthly positive number of the Warrenton and Wytheville laboratories and the monthly positive percentage of the Wytheville laboratory were transformed by taking the square roots and natural logs, respectively, in order to have satisfactory residual plots.

1. Harrisonburg laboratory

The results of the regression computations for the number of samples positive for salmonella cultures appear in Table 18.

Table 18. REGRESSION ANALYSIS OF THE MONTHLY NUMBER OF SALMONELLA POSITIVE SAMPLES AT THE HARRISONBURG LABORATORY

Variable	Coefficient	SE	p-values
CONSTANT	-5.92	3.21	0.07
HUMID ^a	0.09	0.04	0.025
LAGVALUE ^b	0.38	0.12	0.002
n = 58	R ² = 0.27	s = 2.3	Adj.R ² =0.24

^aThe average percent relative humidity of that month

^bThe number of positive samples in the previous month.

The proportion of the monthly number of positive samples explained by the model was 27%. The overall model p-value was 0.0002, suggesting that the prediction was warranted. The standard error of the residuals was estimated to be 2.27 samples. There was approximately one more positive sample for every additional 10% of the relative humidity and this increment was statistically significant at the 5% level of significance. This additive effect held for every fixed level of the number of positive samples in the previous month. The LAGVALUE significantly improved the prediction of the number of positive samples once HUMID was already in the model.

The results of the regression computations for the percentage of samples positive for salmonella cultures appear in Table 19.

Table 19. REGRESSION ANALYSIS OF THE MONTHLY PERCENTAGE OF SALMONELLA POSITIVE SAMPLES AT THE HARRISONBURG LABORATORY

Variable	Coefficient	SE	p-values
CONSTANT	-44.48	19.47	0.026
HUMID ^a	0.76	0.24	0.003
n = 58	R ² = 0.15	s = 14.1	Adj.R ² =0.13

^aThe average percent relative humidity of that month

The interpretation is that the monthly average relative humidity provided significant information (p=0.003) for predicting the monthly percentage of positive samples, although only 15% of variation in the percentage of positive samples can be explained by the relative humidity.

2. Lynchburg laboratory

No variable stayed in the model at the 0.10 level for the regression of the number of positives and no variable met the 0.25 significance level for entry into the model for the percentage of positives. Thus, the regression analysis was stopped at the Stepwise Procedure.

3. Warrenton laboratory

The monthly number of positive samples was transformed to its square root values and the final regression model is given by the Table 22.

Table 20. REGRESSION ANALYSIS OF THE SQUARE ROOT OF THE MONTHLY NUMBER OF SALMONELLA POSITIVE SAMPLES AT THE WARRENTON LABORATORY

Variable	Coefficient	SE	p-values
CONSTANT	-0.12	0.22	0.60
RAIN ^a	0.11	0.04	0.02
SEASON1 ^b	0.03	0.25	0.90
SEASON2 ^c	0.04	0.26	0.89
SEASON3 ^d	0.56	0.25	0.03
n = 36	R ² = 0.27	s = 0.53	Adj.R ² =0.18

^aThe total precipitation (in inches) of that month

^bIndicator variable for spring season

^cIndicator variable for summer season

^dIndicator variable for fall season

The overall p-value of the model was 0.04. The prediction of positive samples was statistically improved with the precipitation ($p=0.02$). At a fixed level of RAIN, the spring, summer, and fall seasons had 0.03, 0.04 and 0.56 more predicted positive samples than the winter, respectively, but only the effect of fall season was significant ($p=0.03$). The interaction between the precipitation and the seasons did not seem to be statistically significant. The model explained about 27% of the variation in the response variables. Without indicator variables in the model, RAIN would give nonsignificant prediction ($p=0.07$).

4. Wytheville laboratory

The dependent variables of this laboratory were also transformed by taking the square root. For the number of samples positive for salmonella cultures (y_i), the prediction equation is given in the Table 21.

TABLE 21. REGRESSION ANALYSIS OF THE SQUARE ROOT OF THE MONTHLY NUMBER OF SALMONELLA POSITIVE SAMPLES AT THE WYTHEVILLE LABORATORY

Variable	Coefficient	SE	p-values
CONSTANT	3.32	1.13	0.006
HUMID ^a	-0.03	0.01	0.044
PRERAIN ^b	-0.12	0.07	0.012
n = 32	$R^2 = 0.21$	$s = 0.64$	Adj. $R^2=0.16$

^aThe percent relative humidity of that month

^bThe total precipitation (in inches) of the previous month

The overall p-value of the model was statistically significant ($p=0.03$); this meant that the relative humidity provided significant help in predicting the square root values of the number of positive samples. The coefficient of determination is 0.21 and the standard error is 0.64.

The equation for estimating the percentage of salmonella positive samples is as follows (Table 22):

TABLE 22. REGRESSION ANALYSIS OF THE NATURAL LOG OF THE MONTHLY PERCENTAGE OF SALMONELLA POSITIVE SAMPLES AT THE WYTHEVILLE LABORATORY

Variable	Coefficient	SE	p-values
CONSTANT	7.77	3.05	0.02
HUMID ^a	-0.08	0.04	0.03
n = 32	R ² = 0.14	s = 1.74	Adj.R ² =0.12

^aThe average percent relative humidity of that month

The relative humidity significantly estimated the natural log values of the monthly percentage of positive samples (p=0.03). The average relative humidity estimated the dependent variable with a standard error of 1.74 and explains only 14% of the variation in the dependent variable.

Evaluation of the Model Error Terms

According to the model assumptions, the model error terms or residuals must be uncorrelated, have zero means, constant variances, and normal distributions. The presence of serial correlation in the residuals was detected by the Durbin-Watson test. The correlated error terms were eliminated by adding the lagging values of the dependent variables in the regression models. The residuals analysis revealed that the residuals of the Harrisonburg laboratory data (percentage) were not normally distributed; however, its stem-leaf plot was close to normal distribution. The residuals from the final models of the other laboratories' data appeared to be normally distributed. The fact that the prevalence of having positive samples was rare and, that most of the time the monthly number of positive samples was zero accounted for the departure from the normal distribution. It was unlikely that the model was involved in other more complex functions, since the plots between the dependent variables and the weather parameters did not have any specific trend. The final models were accepted based on the residual plots that were not extremely deviated from normality and showed acceptable variance distributions.

Chapter 5.

Discussion

Potential Source of Bias

Finding the true prevalence of salmonellosis in cattle for this type of study could be done by randomly selecting cattle from a large population of participating farms, and then regularly collecting and testing specimens from those cattle for several years so that the correlation with the weather can be analyzed. This procedure would be expensive and laborious. Therefore, for this preliminary investigation, data from regional diagnostic laboratories were used. Although the number of positive samples at a laboratory is not the true prevalence or incidence of salmonellosis, it reflects, in a reducing scale, the occurrence of the disease within the cattle population in that area. This study used the number of positive samples as an index for the prevalence of the disease. The method of assuming that recorded data represents the true disease occurrence in a population has been used previously in a study using the number of pneumonic animals from abattoirs⁵² and a study using records of lameness from private practices.¹⁰²

There may be some biases by using the data from records of diagnostic laboratories. The validity of the results may be reduced by the biases. Inconsistency of veterinarians who collected and submitted bovine samples for salmonella culture may have resulted in selection bias; for example, veterinarians may collect samples from suspicious animals more often at a particular time of the year. Selection bias also may occur if there was any situation, other than the change in prevalence of salmonellosis, that caused a high number of submitted samples at a particular laboratory in a particular time. For example, the inconvenience of transportation of samples to one laboratory may result in more samples being submitted to other laboratories. Calving season may be a confounding bias causing nonhomogeneity among the positive samples. Calves are at greater risk compared to adult cattle.⁴⁸ When there is a higher number of calves in the population, the number of positive samples at a laboratory may increase as a consequence. The correlation coefficients between the weather parameters and the positive samples may be partially affected by the selection bias and confounding bias. The selection bias cannot be controlled in this study because veterinarian's consistency is not measurable and there was no information on why a sample was submitted at a particular laboratory. A variable, the percentage of salmonella positive samples, was introduced as an option if there was bias due to inconsistency of veterinarians. The confounding bias can be controlled by accounting for the number of calves in the population in the statistical analysis; however, it was not controlled in this study because the calving distribution was not known.

There was a question raised during the study as to whether the monthly number of samples submitted affects the monthly number of positive samples. During a month that a veterinarian collects more samples than usual, the bacterial culture results may yield a higher number of positive samples although the prevalence of the disease still remains constant. Therefore, the monthly percentage of positive samples was used in an attempt to account for this effect. The disadvantage of the percentage is that if the monthly number of submitted samples is low, only a few positive cultures will account for a high

proportion of the total number of submitted samples; as a result, fluctuation of the monthly percentage of positive samples will be dramatic. Therefore, both variables, the number and the percentage of salmonella positive samples, were used in the analysis. The percentage of bovine samples positive for salmonella culture is not comparable between laboratories because different denominators were used for calculation of the percentage for each laboratory. The percentage of salmonella positive samples was high at the Harrisonburg laboratory because only bovine samples that were specifically requested for *Salmonella* (indicated that *Salmonella* were suspected) were cultured. The other three laboratories cultured certain bovine samples for *Salmonella* even though the test was not specifically requested.

I could not tell if there were any animals whose specimens were submitted repeatedly because the identification number of most animals was not available in the records. Also, the collected data did not include all the positive samples isolated in the state of Virginia, since data from two other regional diagnostic laboratories and data from private laboratories were not included in this study.

Choice of Analysis Method

The separate analysis for each laboratory was reasonable because the preliminary data exploration showed different trends in relationships between the weather conditions and positive samples at different laboratories. Combining the data of all laboratories together may make the results not valid for each individual laboratory. There were some factors that may have resulted in a different number and trend of submitted samples and positive samples at each laboratory: for example, the difference of livestock population in the areas that the laboratories were located; the different criteria by which a submitted bovine sample would be cultured for *Salmonella*; and the differences of veterinarians, culture protocols, and microbiologists. The studied laboratories used different culture methods for isolation of *Salmonella* in submitted samples (see Appendix 2). The difference of culture methods may result in the different number of isolations among laboratories but do not affect the trend of isolations at each laboratory.

The Stepwise Procedure, which is widely used, was chosen for this study since it is better than the Forward selection and Backward elimination. There were nine variables of interest, which were also used in the correlation analysis, for the variable selection. The effect of the season of the year on the cattle environment and management may contribute to the amount of positive samples, so adding the indicator variables in the regression model was necessary unless it caused multicollinearity. For the Warrenton laboratory, the precipitation alone would not give any significant prediction from the regression model until the indicator variables for season were added.

Interpretation of Results

The most common serotype isolated at the studied laboratories was *S. typhimurium* (75.2%), which was also the most frequently isolated serotype in calves in the United States according to a study of the U.S. Department of Agriculture in 1991-1992.⁹³ It was a surprise to me that *S. dublin*, which was described as one of the most common serotypes

in cattle^{44,71,95} appears to be rare in the state of Virginia, as it was found in only one of 222 positive samples (0.45%). *Salmonella dublin* may not be common in this area of the country. According to Anderson and Blanchard, *S. dublin* is more prevalent in the western United States.² The annual number of isolations at the Harrisonburg laboratory was higher than at the other laboratories (Table 1) because the Harrisonburg laboratory is located in the area that has the highest number and density of cattle in Virginia (Figure 15). Although I presumed that the reported number of positive samples was a reflection of the prevalence of salmonella infection, the true prevalence was expected to be higher than what was reported because the specimens were usually collected from animals that showed clinical signs. Specimens from carrier animals were unlikely to be collected.

Before the statistical results were calculated, I expected precipitation to positively correlate with the number of positive samples, since wet weather leads to increased susceptibility of salmonellosis in animals.⁴⁴ I never expected a specific direction of a relationship between the amount of positive samples and the temperature or humidity as my literature review did not give conclusive evidence about the effect of them on *Salmonella* and animals. High environmental temperature reduces host resistance because it increases the level of corticosteroids in animals;³⁴ however, winter can have the same effect on animals due to poor nutritional status at this time.¹⁰⁰ The effects of temperature on the organism reported in many studies were not consistent probably due to different types of samples in different experiments; i.e. fresh slurry, feces on pasture, urine, and soil samples. The studies that reported the effects of humidity on the organisms were also not helpful because the response reported in those studies was caused by humidity ranging from 30 to 80% while the humidity in my study stayed about 70-80% all the time.

The Harrisonburg laboratory results have the most precision among the four studied laboratories because the number of observations was almost twice that of the other laboratories. According to the Harrisonburg laboratory data, the number of samples positive for salmonella culture tended to increase when the temperature and relative humidity were increased. The observed higher occurrence of salmonellosis in the summer and fall agreed with the U.S. Department of Agriculture study that found higher prevalence of salmonellosis in dairy calves in summer.⁹³ The same trend was also described in Hinton's study that used a two-year survey and found more cases of salmonella enteritis in cattle between May and November.³⁶ The increase in isolations of *Salmonella* in ground and surface water samples was also found to correlate with warm temperatures.²¹ However, this positive correlation found at the Harrisonburg laboratory was not consistent with the results of the other laboratories. The other laboratories' data showed that positive samples tend to be fewer when the temperature and relative humidity were high. This indicates the existence of interaction between the factors involved at laboratories or in the areas from which samples were submitted and the weather conditions. One of the factors involved with laboratories and interacted with the weather may be the difference in the type of livestock in the areas that the laboratories are located. The Harrisonburg laboratory located in an area with a high concentration of dairy cattle (Figure 13). Although not as seasonal as for beef cattle, the number of dairy cows calving in this region tends to increase in August, September, and October resulting in a high number of calves during the summer and fall. A neonatal calf is highly

susceptible to salmonellosis due to lack of protective acidity in the abomasum and lack of competing flora in the gut.² Consequently, the incidence of salmonellosis and the prevalence of shedding *Salmonella* in feces are also high in the summer and fall. Additionally, cattle stress due to late pregnancy is high in June, July, and August. This may result in higher susceptibility and higher incidence of salmonellosis in a herd during June-August. Cattle in the area of the Wytheville laboratory are mostly beef cattle (Figure 14). Beef cattle has its calving season either in November and December or March, April, and May. The high number of calves in winter and spring may cause a high incidence of salmonellosis among beef livestock at this time. Stress of late pregnancy, which occurs mostly in late fall and winter, may cause higher incidence of salmonellosis in pregnant cows and heifers as well. New beef calves are usually brought on farms during October and November. Therefore, the peak incidence of salmonellosis among transported beef calves may occur in the winter.

The percentage of positive samples had the same trend as the number of positive samples for every laboratory suggesting that the results observed were not do only to an increase in the number of samples submitted. The correlation coefficients of the monthly positive number at the Harrisonburg laboratory with the temperature and relative humidity were considered low ($r_s=0.25, 0.37$, respectively); although they were significant at a 0.05 level. A study of correlation between lameness incidence in cows and rainfall reported the degree of coefficients ranging from 0.07-0.85.¹⁰² Subsequently, a study in sheep reported a correlation coefficient for pneumonia occurrence and rain/wind-chill factor as high as 0.92.⁵² The low correlation coefficients indicated weak relationships between the monthly reported occurrence and the weather parameters; consequently, the regression models accounted for only a small proportion of variations among positive samples.

The survival time of salmonella organisms in the environment could play a role on the incidence of disease. Wray and Sojka pointed out in their review that *Salmonella* have longer environmental survival time in the summer compared to the other seasons.¹⁰⁹ This may account for the higher number of positive bovine samples in the summer. Another potential reason for the seasonal incidence of positive samples was the grazing season. It may cause a peak of salmonellosis in adult cattle in summer and fall because of contaminated pastures during the grazing season.¹⁰⁹ Heat stress was less likely to cause high occurrences in the summer and fall according to a study in Texas hogs that found no difference between positive isolations in summer and winter.¹⁴

The results from the Harrisonburg laboratory, which were the most precise, showed stronger correlations of the temperature with the number of positive samples reported one month later. Similarly a correlation between pneumonia in sheep and wind speed was obtained.⁵² It was likely that the effects from the temperature in the previous month affected animals or allowed environmental proliferation of *Salmonella* which resulted in more positive samples than the temperature in the same month.

The monthly number of positive samples at the Harrisonburg laboratory was positively correlated to the temperature and relative humidity partly because of unusual outbreaks in the summer of 1994 in the northwestern part of Virginia. Hence, when the data of 1994 were removed, the correlation coefficients were reduced and so was the significance of the correlation. However, the direction of the correlation coefficients

were still positive without the data of 1994 and still more reliable than the results from other laboratories because of the higher number of observations.

It is remarkable that there was no significant association between the number of positive samples and the precipitation found at any laboratory despite a review suggested that wet weather causes higher susceptibility to salmonellosis in cattle.⁴⁴ This study cannot clearly identify whether temperature or relative humidity caused the significant correlation with the positive samples because temperature and relative humidity are highly correlated. Identifying the actual variable which accounted for the significant correlations would probably not be possible because temperature and relative humidity are highly correlated in this region.

The presence of serial correlation to the number of positive samples suggested that having control on salmonella positive animals is important to the spread of the disease. The regression results support this idea since the lagging values of positive samples shared a bigger portion on the variation of the number of positive samples than did the relative humidity (Table 18).

This study has demonstrated that the fluctuation of the number of positive samples is significantly correlated to the temperature and the relative humidity. However, the degree of correlation is not strong and does not necessarily demonstrate the causal relationship. The direction of correlation between weather conditions and the disease's occurrence lacks consistency due to unknown extraneous factors. Additional studies are necessary in order to identify the factors that may cause this inconsistency; for instance, the difference between beef and dairy farm management, calving season, stocking season, and other health problems.

The results of this study support the hypothesis which states that the weather condition is a potential risk factor for the occurrence of salmonellosis in cattle. According to the statistical results obtained from the data set which had the highest number of observations, bovine salmonellosis is likely to occur during the summer and fall in the region of Virginia near the Harrisonburg laboratory. The weather parameters that are associated with disease occurrence are the temperature and relative humidity. This result suggested that the temperature and relative humidity should be controlled, in future studies, as factors that relate to the increase of salmonellosis in cattle. The temperature and relative humidity can be controlled either experimentally or statistically. For example, a study that evaluates preventive measures of salmonellosis should consider applying every measure at the same period of the year or treating temperature and relative humidity as covariates in statistical analysis. A study that investigates the effect of a factor on incidence of salmonellosis should have the temperature and relative humidity controlled as well.

Chapter 6.

Summary

Weather conditions were considered to be potential risk factors for bovine salmonellosis as there are studies demonstrating the effects of weather on the growth and survival of salmonella organisms.^{20,23,41,109} Additionally, it is generally known that weather conditions have an influence on cattle stress and result in lower immune status.³⁴ This study was designed to investigate the correlation between the prevalence of samples positive for salmonella culture and the weather parameters: temperature, precipitation, and relative humidity. Cattle records from four out of six regional diagnostic laboratories in the state of Virginia were examined. The number of samples positive for salmonella culture was recorded over three years by three laboratories and over five years by one laboratory. The weather parameters were collected for the same regions; the monthly average temperature, monthly total precipitation and monthly average relative humidity. A correlation analysis was performed using the Spearman's rank correlation coefficient. Two out of four studied laboratories, the Harrisonburg and Wytheville laboratories, had significant correlation coefficients between the monthly positive number and the monthly average temperature. The Harrisonburg laboratory's highest correlation coefficient was found between the monthly average temperature and the monthly number of positive samples reported one month later ($r_s=0.30$, $p=0.02$). The Wytheville laboratory's highest correlation coefficient was negative and was found when using the average temperature of the month corresponding to the number of positive samples ($r_s=-0.38$, $p=0.03$).

The significant correlation coefficients between the monthly average relative humidity and the monthly number of positive samples were obtained from the same laboratories that had significant correlations between the positive samples and the temperature. Similar to the correlation with the temperature, the directions of correlations from those two laboratories were opposite. The Harrisonburg laboratory's highest correlation coefficient was 0.37 and that of Wytheville was -0.39; both of them were found between the average relative humidity and the positive number of salmonella culture reported in the same month. There was no significant correlation between the monthly number of samples positive for salmonella culture and the monthly total precipitation ($p > 0.05$).

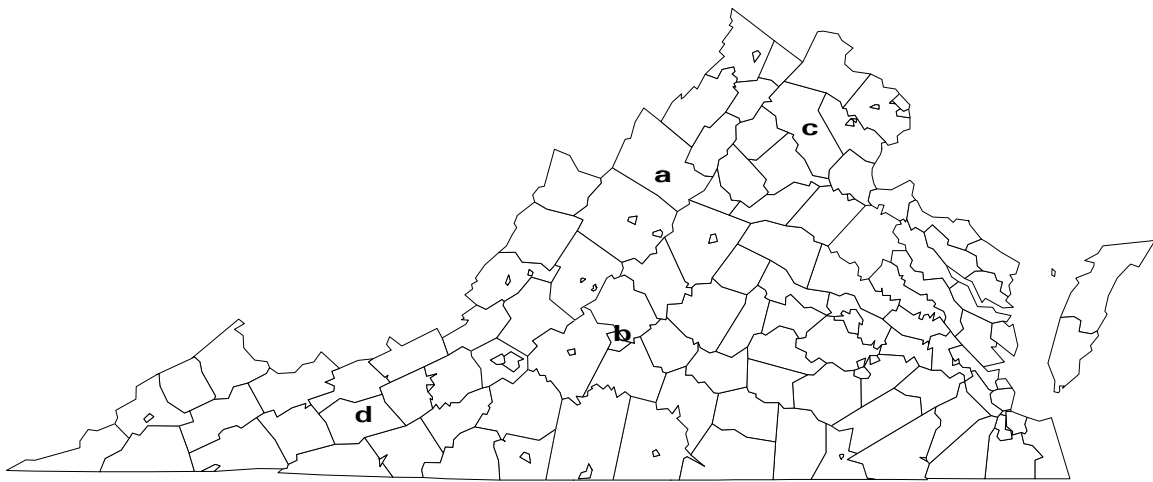
The fluctuation of cattle salmonellosis occurrence may be the result of a combination of the survival of the salmonella organisms, which varies with temperature and humidity in the environment,^{23,41,109} and the host resistance that is altered by different weather conditions.^{34,44,100} Lack of consistency between the correlations obtained from different areas suggests that the effect of weather conditions on the development of bovine salmonellosis depends on factors specific to those regions. Some factors that may account for differences in the correlations observed are livestock types (beef and dairy), calving period, and transportation of calves. It is also possible that the inconsistent results are only because of a lack of enough observations since the largest data set was collected only for a five year period.

Although causality cannot be assumed, the results of this study show that the weather conditions were associated with the number of positive cultures in two of four diagnostic

laboratories. The temperature and relative humidity should be considered in future epidemiological studies that are involved in investigating bovine salmonellosis occurrence in different conditions.

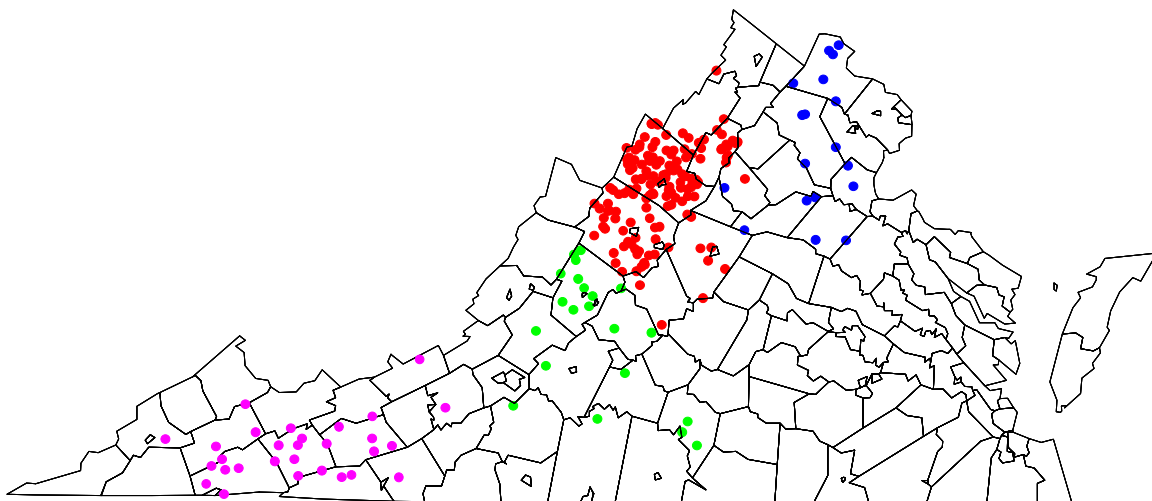
Appendix 1.

Locations of Salmonella Positive Samples and Laboratories



- a** : Harrisonburg Laboratory and Dale Enterprise Station
- b** : Lynchburg Laboratory and Lynchburg WSO Airport Station
- c** : Warrenton Laboratory and Warrenton 3 SE Station
- d** : Wytheville Laboratory and Wytheville 1 S Station

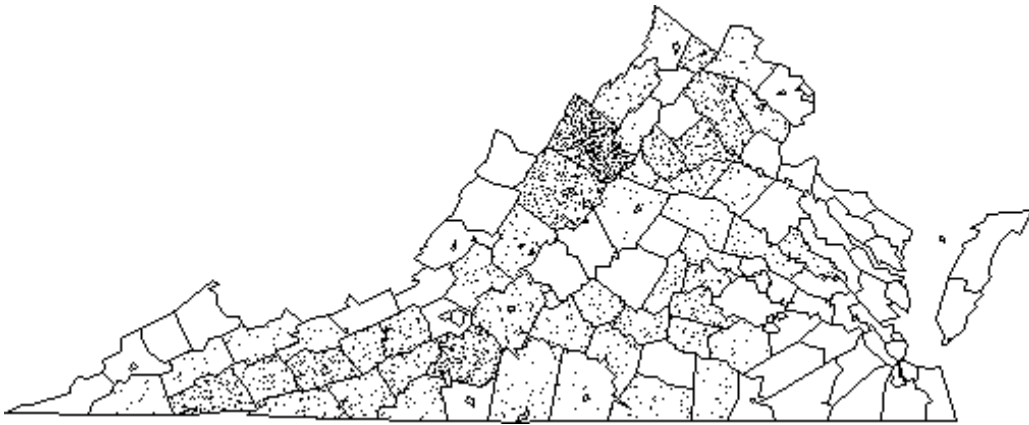
Figure 11. LOCATION OF STUDIED LABORATORIES AND WEATHER STATIONS



1 dot = 1 positive sample

Harrisonburg---■
Lynchburg-----■
Warrenton-----■
Wytheville-----■

Figure 12. LOCATIONS BY COUNTY OF BOVINE SAMPLES POSITIVE FOR SALMONELLA CULTURE AT THE HARRISONBURG, LYNCHBURG, WARRENTON, AND WYTHEVILLE LABORATORIES



State total = 130,000
 1 dot = 50 head
 Counties with less than 500 head are blank

(Data from Virginia Agricultural Statistics, 1994)

Figure 13. VIRGINIA MILK COWS: NUMBER ON FARMS, JANUARY 1, 1994



State total = 700,000
 1 dot = 50 head
 Counties with less than 500 head are blank

(Data from Virginia Agricultural Statistics, 1994)

Figure 14. VIRGINIA BEEF CATTLE: NUMBER ON FARMS, JANUARY 1, 1994



Averaged state total, 1991-1994 = 1,742,500
1 dot = 200 head
Counties with less than 500 head are blank

(Data from Virginia Agricultural Statistics, 1991-1994)

Figure 15. VIRGINIA CATTLE (BEEF AND DAIRY): AVERAGE NUMBER ON FARMS, 1991-1994

Appendix 2.

Salmonella Isolation Techniques

Harrisonburg Regional Laboratory

1. Place fecal or tissue swabs in Selenite broth then incubate overnight at 35 °C.
2. Transfer the broth to Hektoen agar and EMB or MacConkey's agar plates. Look for colonies that are H₂S positive. In MacConkey's agar plates, typical colonies have light color with rough edges.
3. Suspicious colonies are identified by use of TSI slant and API 20E test strip.
4. Positive isolations are sent to National Veterinary Services Laboratory, Ames, Iowa for serotyping.

Lynchburg Regional Laboratory

1. Place 1 g. of feces or gut loop in 100 ml. Selenite broth then incubate for 18-24 hours. at 37 °C.
2. Place a loopful of incubated broth on Hektoen agar. Streak for isolation then incubate at 37 °C for 18-24 hours. Look for colonies that are green with black center.
3. If negative results occur, leave the Selenite broth at room temperature for five days then repeat the streak on Hektoen agar again.
4. Suspicious colonies from Hektoen agar are identified by using gram stain, API 20E test strip, TSI agar and LIA agar.
5. *Salmonella* colonies are sent to National Veterinary Services Laboratory, Ames, Iowa for serotyping.

Warrenton Regional Laboratory

- 1) Inoculate fecal or tissues swabs in Selenite broth, TSA with 5% sheep blood agar, Hektoen agar, and MacConkey's agar.
- 2) Incubate Selenite broth overnight at 37 °C. Inoculate broth onto Hektoen agar
- 3) Incubate all plates at 37 °C in non-CO₂ condition.
- 4) Look for non-fermentor colonies or H₂S positive colonies.
- 5) Identify suspicious colonies by using API 20E test strip.
- 6) *Salmonella* colonies are sent to National Veterinary Services Laboratory, Ames, Iowa for serotyping.

Wytheville Regional Laboratory

1. Culture of tissues

a. Direct plating

- 1) Sear surface of organ to be cultured.
- 2) Cut with sterile scissors and swab into tissue.
- 3) Smear swab on Hektoen Enteric Agar and streak out with sterile inoculating loop.
- 4) Examine plate for typical *Salmonella* colonies after 24 hours of incubation at 37 °C (typical colonies are green and usually have a dark center).

b. Enrichment culture

- 1) Place tissue or fecal swab in 10 ml. Selenite Cystine Broth.
- 2) Incubate Selenite broth at 37 °C for 18-24 hours.
- 3) Plate broth onto Hektoen Enteric Agar.
- 4) Incubate for 24 hours at 37 °C.
- 5) Examine plate for typical *Salmonella* colonies.

2. Culture of feces

a. Direct plating

- 1) Fecal swabs can be plated directly onto Hektoen Agar.
- 2) Follow above technique.

3. Biochemical analysis

- a. Suspicious colonies are identified by use of bioMerieux Vitek 20E system.
- b. Colonies that prove to be *Salmonella* are placed on nutrient agar.
- c. Isolates are then shipped to National Veterinary Services Laboratory, Ames, Iowa for serotyping.

References

1. Aitken, M. M., G. A. Hall and P. W. Jones. 1978. Investigation of a cutaneous delayed hypersensitivity response as a means of detection *Salmonella dublin* infection in cattle. *Res. Vet. Sci.* 24(3): 370-374.
2. Anderson, M., and P. Blanchard. 1989. The clinical syndromes caused by *Salmonella* infection. *Vet. Med.* 84(8): 816-819.
3. Anderson, J., B. P. Smith and J. T. Ulrich. 1991. Vaccination of calves with a modified bacterin or oil-in-water emulsion containing alkali-detoxified *Salmonella typhimurium* lipopolysaccharide. *Am. J. Vet. Res.* 52(4): 596-601.
4. Armstrong, D. V. 1994. Symposium: Nutrition and heat stress: Heat stress interaction with shade and cooling. *J. Dairy Sci.* 77(7): 2044-2050.
5. Beumer, R. R., E. Brinkman and F. M. Rombouts. 1991. Enzyme-linked immunoassays for the detection of *Salmonella spp.*: A comparison with other methods. *Int. J. Food Micro.* 12(4): 363-374.
6. Breazile, J. E. 1988. The physiology of stress and its relationship to mechanisms of disease and therapeutics. *Vet. Clin. North Am. Food Pract.* 4(3): 441-578.
7. Butler, D. G. 1986. Bovine salmonellosis. *Proc. Annu. Conv. Am. Assoc. Bovine Pract.* 18: 14-19.
8. Cherrington, C. A. and J. H. J. Huis in't Veld. 1993. Comparison of classical isolation protocols with a 24 h screen to detect viable salmonellas in faeces. *J. Appl. Bact.* 75(1): 65-68.
9. Clark, M. A., M. A. Jepson, N. L. Simmons and B. H. Hirst. 1994. Preferential interaction of *Salmonella typhimurium* with mouse Peyer's patch M cells. *Res. Microbiol.* 145(7):543-552.
10. Clarke R. C. and C. L. Gyles. 1993. *Salmonella*. In *Pathogenesis of Bacterial Infections in Animals*, ed. C. L. Gyles and C. O. Thoen. Ames, Iowa: Iowa State University Press, pp. 133-153.
11. Corrier, D. E., C. W. Purdy and J. R. DeLoach. 1990. Effects of marketing stress on fecal excretion of *Salmonella spp.* in feeder calves. *Am. J. Vet. Res.* 51(6): 866-869.
12. Cox, C. P. 1987. *A handbook of introductory statistical methods*. New York: John Wiley & Sons, Inc.

13. Cullor, J. S. 1995. Common pathogens that cause foodborne disease: Can they be controlled on the dairy. *Vet. Med.* 90(2): 185-194.
14. Currier, M., M. Singleton, J. Lee, and D. R. Lee. 1986. Salmonella in swine at slaughter: Incidence and serovar distribution at different seasons. *J. Food Prot.* 49(5): 366-368.
15. Curtiss III, R., S. M. Kelly and J. O. Hassan. 1993. Live oral avirulent salmonella vaccines. *Vet. Micro.* 37(3-4): 397-405.
16. D'Aoust J. Y. 1991. Pathogenicity of foodborne salmonella. *Int. J. food Micro.* 12(1):17-40.
17. D'Aoust, J. Y., A. M. Sewell and D. W. Warburton. 1992. A comparison of standard cultural methods for the detection of foodborne salmonella. *Int. J. Food Micro.* 16(1): 41-50.
18. Davies, R. H. and C. Wray. 1994. Evaluation of a rapid cultural method for identification of salmonellas in naturally contaminated veterinary samples. *J. Appl. Bact.* 77(3): 237-241.
19. Davies, R. H. and C. Wray. 1996. Seasonal variations in the isolation of *Salmonella typhimurium*, *Salmonella enteritidis*, *Bacillus cereus* and *Clostridium perfringens* from environmental samples. *Zentralbl Veterinarmed [B]*. 43(2): 119-127.
20. Dinter, P. S. and W. Muller. 1988. The tenacity of bacteria in the airborne state : Tenacity of airborne *S. senftenberg*. *Zentralbl. Bakteriol Mikrobiol Hyg B.* 186(3): 278-288.
21. El-Figi, K. A. 1990. Epidemiological and microbiological evaluation of enteric bacteria waterborne diseases in coastal areas of south carolina. *Ph.D. Diss., University of South Carolina*.
22. Fathy, E. E. and E. H. Marth. 1992. Dairy food: Salmonellae, salmonellosis and dairy foods: A review. *J. Dairy Sci.* 75(9): 2327-2343.
23. Findlay, C. R. 1972. The persistence of *Salmonella dublin* in slurry in tanks and on pasture. *Vet. Rec.* 91(10): 233-235.
24. Fone, D. L. and R. M. Barker. 1994. Associations between human and farm animal infections with *Salmonella typhimurium* DT104 in herefordshire. *Commu. Dis. Rep. CDR. Wkly.* 4(11): 136-140.
25. Freund R. J. and P. D. Minton. 1979. *Regression method: A tool for data analysis*. New York: Marcel Dekker, Inc .

26. Gay, J. M., D. H. Rice and J. H. Steiger. 1994. Prevalence of fecal *Salmonella* shedding by cull dairy cattle marketed in Washington state. *J. Food. Prot.* 57(3): 195-197.
27. Gay, J. M. and M. E. Hunsaker. 1993. Isolation of multiple salmonella serotypes from a dairy two years after a clinical salmonellosis outbreak. *J. Am. Vet. Med. Assoc.* 203(9): 1314-1320.
28. Giles, N., S. A. Hopper and C. Wray. 1989. Persistence of *S. typhimurium* in a large dairy herd. *Epidemiol. Infect.* 103(2): 235-241.
29. Glock, R. D. and K. J. Schwartz. 1993. Salmonellosis: A growing problem for food animal producers. *Large Anim. Vet.* 48(1): 12-14.
30. Groothuis, D. G. and A. S. J. P. A. M. van Miert. 1987. Salmonellosis in veal calves: Some therapeutic aspects. *Vet. Quarterly.* 9(1): 91-96.
31. Gunst, R. F. and R. L. Mason. 1980. *Regression analysis and its application*. New York: Marcel Dekker, Inc.
32. Guthrie, R. K. 1992. *Salmonella*. Florida: CRC Press, Inc.
33. Hall, G. A., D. J. Reynolds, K. R. Parsons, A. P. Bland and J. H. Morgan. 1988. Pathology of calves with diarrhoea in southern Britain. *Res. Vet. Sci.* 45(2): 240-250.
34. Hancock, D. 1984. Effects of the environment on neonatal dairy calf health. *Proc. Annu. Conv. Am. Assoc. Bovine Pract.* 16: 85-87.
35. Hardman, P. M., C. M. Wathes and C. Wray. 1991. Transmission of salmonellae among calves penned individually. *Vet. Rec.* 129(15): 327-329.
36. Hinton, M. 1986. Salmonella abortion in cattle. *Vet. Annual.* 26: 81-89.
37. Hoorfar, J. and V. Bitsch. 1995. Evaluation of an O-antigen for screening cattle herds for *Salmonella typhimurium*. *Vet. Rec.* 137(15): 374-379.
38. House, J. K., B. P. Smith, G. W. Dilling and L. D. Roden. 1993. Enzyme-Linked Immunosorbent Assay for serologic detection of *Salmonella dublin* carriers on a large dairy. *Am. J. Vet. Res.* 54(9): 1391-1399.
39. Jones, P. W. 1992. Salmonellosis. In *Bovine Medicine: Diseases and Husbandry of Cattle*, 2nd. ed., ed. A. H. Andrews, R. W. Blowey, H. Boyd and R. G. Eddy. Oxford: Blackwell Scientific Publications. pp. 181-193.
40. Jones, P. W., P. Collins, G. T. H. Brown and M. Aitken. 1982. Transmission of *Salmonella mbandaka* to cattle from contaminated feed. *J. Hyg.* 88: 255-263.

41. Kearney, T. E., M. J. Larkin and P. N. Levett. 1993. The effect of slurry storage and anaerobic digestion on survival of pathogenic bacteria. *J. Appl. Bacteriol.* 74(1): 86-93.
42. Kleinbaum, D. G., L. L. Kupper, and K. E. Muller. 1988. *Applied regression analysis and other multivariable methods*, 2nd. edition., Boston, Mass: PWS-KENT Publishing Company.
43. Lance, S. E., G. Y. Miller, D. D. Hancock, P. C. Bartlett and L. E. Heider. 1992. Salmonella infections in neonatal dairy calves. *J. Am. Vet. Med. Assoc.* 201(6): 864-868.
44. Lax, A. J., P. A. Barrow, P. W. Jones and T. S. Wallis. 1995. Current perspectives in salmonellosis. *Br. Vet. J.* 151:351-376.
45. Lee, L. A., N. D. Puh, E. K. Maloney, N. H. Bean and R. V. Tauxe. 1994. Increase in antimicrobial-resistant *Salmonella* infections in the United States, 1989-1990. *J. Infect. Dis.* 170(1):128-34.
46. Lindgren, S. W., I. Stojiljkovic and F. Heffron. 1996. Macrophage killing is an essential virulence mechanism of *Salmonella typhimurium*. *Proc. Natl. Acad. Sci. USA.* 93(9): 4197-4201.
47. Mackey, B. M. and A. L. Kerridge. 1988. The effect of incubation temperature and inoculum size on growth of salmonellae in minced beef. *Int. J. Food Microbiol.* 6(1): 57-65.
48. McDonough, P. L. 1986. Epidemiology of bovine salmonellosis. *Proc. Annu. Conv. Am. Assoc. Bovine Pract.* 18: 169-173.
49. McDonough, P. L. 1995. Salmonellosis: Diagnostic approach to disease control and epidemiology in the bovine animal. *Proc. Annu. Conv. Am. Assoc. Bovine Pract.* 27: 61-68.
50. McDonough, S. P., C. L. Stull and B. I. Osburn. 1994. Enteric pathogen in intensively reared veal calves. *Am. J. Vet. Res.* 55(11): 1516-1520.
51. McLaren, I. M. and C. Wray. 1991. Epidemiology of *Salmonella typhimurium* infection in calves: Persistence of salmonellae on calf units. *Vet. Rec.* 129(21): 461-462.
52. McLlroy, S. G., E. A. Goodal, R. M. McCracken and D. A. Stewart. 1989. Rain and windchill as factors in the occurrence of pneumonia in sheep. *Vet. Rec.* 125(4): 79-82.

53. Morisse, J. P. and J. P. Cotte. 1994. Evaluation of some risk factors in bovine salmonellosis. *Vet. Res.* 25(2-3): 185-191.
54. Morter, R. L., C. H. Armstrong, H. E. Amstutz and H. L. Thacker. 1989. Systemic salmonellosis in mature beef cows. *J. Vet. Diagn. Invest.* 1(1): 22-24.
55. Myer, H., H. Koch, U. Methner and G. Steinbach. 1993. Vaccines in salmonellosis control in animals. *Zbl. Bakt.* 278(2-3): 407-415.
56. Myers, R. H., 1990. *Classical and modern regression with applications*, 2nd. ed. Boston, Mass: PWS-KENT Publishing Company.
57. Nastasi, A., C. Mammina and M. R. Villafrate. 1993. Epidemiology of *Salmonella typhimurium*: Ribosomal DNA analysis of strains from human and animal sources. *Epidemiol. Infect.* 110(3): 553-565.
58. Nolan, L. K., C. W. Giddings, E. W. Boland, D. J. Steffen, J. Brown and A. Misek. 1995. Detection and characterization of *Salmonella typhimurium* from a dairy herd in North Dakota. *Vet. Res. Comm.* 19(1): 3-8.
59. Oosterom, J. 1991. Epidemiological studies and proposed preventive measures in the fight against human salmonellosis. *Int. J. Food. Microbiol.* 12(1): 41-52.
60. Pacer, R. E., J. S. Spika, M. C. Thurmond, N. Hargrett-Bean, and M. E. Potter. 1989. Prevalence of *Salmonella* and multiple antimicrobial-resistant *Salmonella* in California dairies. *J. Am. Vet. Med. Assoc.* 195(1): 59-63.
61. Palmer, J. E., R. H. Whitlock, C. E. Benson, J. L. Becht, D. D. Morris and H. M. Acland. 1985. Comparison of rectal mucosal cultures and fecal cultures in detecting *Salmonella* infection in horses and cattle. *Am. J. Vet. Res.* 46(3): 697-698.
62. Peel, J. E., C. Kolly, B. Siegenthaler and S. R. Martinod. 1990. Prophylactic effects of recombinant bovine interferon- α_1 on acute *Salmonella typhimurium* infection in calves. *Am. J. Vet. Res.* 51(7): 1095-1099.
63. Pelton, J. A., G. W. Dilling, B. P. Smith and S. Jang. 1994. Comparison of a commercial antigen-capture ELISA with enrichment culture for detection of salmonella from fecal samples. *J. Vet. Diagn. Invest.* 6(4): 501-502.
64. Pelzer, K. D. 1989. Salmonellosis. *J. Am. Vet. Med. Assoc.* 195(4): 456-462.
65. Peters, A. R. 1985. An estimation of the economic impact of an outbreak in a calf rearing unit. *Vet. Rec.* 117(25-26): 667-668.

66. Peters, A. R., C. Wray and T. N. Allsup. 1987. Serological response of calves to a dead salmonella vaccine and its relation to live weight and performance. *Vet. Rec.* 121(4):84-85.
67. Plym-Forshell, L. and I. Ekesbo. 1996. Survival of Salmonellas in urine and dry faeces from cattle: An experimental study. *Acta. Vet. Scand.* 37(2): 127-131.
68. Rawlings, J. O., 1988. *Applied regression analysis: A research tool*. Pacific Grove, California: Wadsworth&Brooks/Cole Advanced Books&Software.
69. Rings, D. M. 1985. Salmonellosis in calves. *Vet. Clin. North Am. Food Anim. Pract.* 1(3): 529-539.
70. Roberts, T. 1989. Human illness costs of foodborne bacteria. *Amer. J. Agr. Econ.* 71(2): 468-474.
71. Robinson, R. A., K. E. Ferris, D. A. Miller, and S. Srinand. 1992. Descriptive epidemiology of salmonella serotypes from cattle in the USA (1982-1991). *Proc. Annu. Conv. Am. Assoc. Bovine Pract.* 25: 15-19.
72. Roden, L. D., B. P. Smith, S. J. Sharon and G. W. Dilling. 1992. Effect of calf age and salmonella bacterin type on ability to produce immunoglobulins directed against salmonella whole cells or lipopolysaccharide. *Am J. Vet. Res.* 53(10): 1895-1899.
73. Rohrbach, B. W., F. A. Draughon, P. M. Davidson and S. P. Oliver. 1992. Prevalence of *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica* and *Salmonella* in bulk tank milk: Risk factors and risk of human exposure. *J. Food Prot.* 55(2): 93-97.
74. Roussel, A. J. Jr. and G. W. Brumbaugh. 1991. Treatment of diarrhea of neonatal calves. *Vet. Clin. North Am. Food. Anim. Pract.* 7(3): 713-726.
75. SAS Institute Inc., 1989. *SAS/STAT User s Guide*, version 6, 4th. ed., volume 2. Cary, NC: SAS Institute Inc.
76. SAS Institute Inc. 1989-1993., *SAS Release 6.10*. Cary, NC: SAS Institute Inc.
77. Sato, Y., M. Schneebeli, K. Matsukawa, H. Chimana, H. Sinsungwe and G. Sato. 1993. Outbreaks of *Salmonella Dublin* infection among calves on a dairy farm applying Salmonella bacterins in Zambia. *J. Vet. Med. Sci.* 55(3): 511-513.
78. Scanlan, C. M. 1988. *Introduction to Veterinary Bacteriology*. Ames, Iowa: Iowa State University Press.
79. Scott, P.1994. Management of a salmonellosis outbreak in a cattle herd. *In Pract.* 16(1): 17-20.

80. Selim, S. A., J. S. Cullor, B. P. Smith, P. Blanchard, T. B. Farver, R. Hoffman, G. Dilling, L. D. Roden and B. Wilgenburg. 1995. The effect of *Escherichia coli* J5 and modified live *Salmonella dublin* vaccines in artificially reared neonatal calves. *Vaccine*. 13(4): 381-390.
81. Smith, B. P., D. G. Oliver, P. Singh, G. Dilling, P. A. Marvin, B. P. Ram, L. S. Jang, N. S. Sharkov, J. S. Orsborn and K. Jackett. 1989. Detection of *Salmonella dublin* mammary gland infection in carrier cows, using an enzyme-linked immunosorbent assay for antibody in milk or serum. *Am. J. Vet. Res.* 50(8): 1352-1360.
82. Smith, B. P., G. W. Dilling, L. D. Roden and B. A. D. Stocker. 1993. Vaccination of calves with orally administered aromatic-dependent *Salmonella dublin*. *Am. J. Vet. Res.* 54(8): 1249-1255.
83. Smith, B. P. and J. House. 1992. Prospects for salmonella control in cattle. *Proc. Annu. Conv. Am. Assoc. Bovine Pract.* 25(1): 67-73.
84. Smith, B. P., L. D. Roden, M. C. Thurmond, G. W. Dilling, H. Konrad, J. A. Pelton and J. P. Picanso. 1994. Prevalence of salmonellae in cattle and in the environment on California dairies. *J. Am. Vet. Med. Assoc.* 205(3): 467-471.
85. Spier, S. J., B. P. Smith, J. S. Cullor, H. J. Olander, L. D. Roden and G. W. Dilling. 1991. Persistent experimental *Salmonella dublin* intramammary infection in dairy cows. *J. Vet. Int. Med.* 5(6): 341-350.
86. Spier, S. J., B. P. Smith, J. W. Tyler, J. S. Cullor, G. W. Dilling and L. D. Pfaff. 1990. Use of ELISA for detection of immunoglobulins G and M that recognize *Salmonella dublin* lipopolysaccharide for prediction of carrier status in cattle. *Am. J. Vet. Res.* 151(12): 1900-1904.
87. Sprowls, R. W. 1995. Bovine salmonellosis. *Proc. Annu. Conv. Am. Assoc. Bovine Pract.* 27: 7.
88. Stadler, P. and J. W. Nesbit. 1990. Salmonellosis in adult dairy cow. *S. Afr. Vet. Assoc.* 61(2): 65-67.
89. Steel, G. D. R. and J. H. Torrie. 1980. *Principles and procedures of statistics: A biometrical approach*, 2nd. ed. New York: McGraw-Hill Book Company.
90. Stone, G. G., R. D. Oberst, M. P. Michael, P. Hays, S. McVey and M. M. Chengappa. 1994. Detection of salmonella serotypes from clinical samples by enrichment broth cultivation-PCR procedure. *J. Clin. Micro.* 32(7): 1742-1749.
91. Tauxe, R. V. 1991. Salmonella: A postmodern pathogen. *J. Food Prot.* 54(7): 563-568.

92. Todd, E. C. D. 1989. Preliminary estimates of costs of foodborne disease in the United States. *J. Food Prot.* 52(8): 595-601.
93. U.S. Department of Agriculture. National Animal Health Monitoring System. 1994. *Salmonella in dairy calves*. Colorado: U.S. Department of Agriculture, National Animal Health Monitoring System.
94. Visser, N. A. P. C., H. J. Breukink, F. G. Zijderveld and P. W. Leeuw. 1987. Enteric infections in veal calves: A longitudinal study on four veal calf units. *Vet. Quarterly.* 9(4): 289-294.
95. Walker, R. L. 1995. *Salmonella dublin* infections in cattle in California. *Proc. Annu. Conv. Am. Assoc. Bovine Pract.* 27: 8-9.
96. Wall, P. G., D. Morgan, K. Lamden, M. Griffin, E. J. Threfall, L. R. Ward, and B. Rowe. 1995. Transmission of multi-resistant strains of *Salmonella typhimurium* from cattle to man. *Vet. Rec.* 136(23): 591-592.
97. Warburton, D. W., B. Bowen, A. Konkle, C. Crawford, S. Durzi, R. Foster, C. Fox, L. Gour, G. Krohn, P. LaCasse, G. Lamontagne, S. McDonagh, V. Arling, J. Mackenzie, E. C. D. Todd, J. Oggel, R. Plante, S. Shaw, N. P. Tiwari, Y. Trottier, B. D. Wheeler. 1994. A comparison of six different plating media used in the isolation of salmonella. *Int. J. Food Micro.* 22(4): 277-289.
98. Warnick, L. D., L. M. Crofton, M. J. Hawkins, K. D. Pelzer, R. A. Ruth, and K. R. Scheel. 1995. Case-control study of clinical salmonellosis in cattle herds. *76th Annual Meeting of the Conference of Research Workers in Animal Diseases. Chicago, IL.*
99. Wathes, C. M., W. A. R. Zaidan, G. R. Pearson, M. Hinton and N. Todd. 1988. Aerosol infection of calves and mice with *Salmonella typhimurium*. *Vet. Rec.* 123(23): 590-594.
100. Webster, A. J. F. 1981. Weather and infectious disease in cattle. *Vet. Rec.* 108(9): 183-187.
101. Wherry, R. J., Sr. 1984. *Contribution to correlation analysis*. Florida: Academic Press, Inc.
102. Williams, L. A., G. J. Rowlands and A. M. Russell. 1986. Effect of wet weather on lameness in dairy cattle. *Vet. Rec.* 118(10): 259-261.
103. Wray, C., J. N. Todd and M. Hinton. 1987. Epidemiology of *Salmonella typhimurium* infection in calves: Excretion of *S. typhimurium* in the faeces of calves in different management systems. *Vet. Rec.* 121(13): 293-296.

104. Wray, C., N. Todd, I. M. McLaren and Y. E. Beedell. 1991. The Epidemiology of salmonella in calves: The role of markets and vehicles. *Epidemiol. Infect.* 107(3): 521-525.
105. Wray, C., N. Todd, I. M. McLaren, Y. Beedell and B. Rowe. 1990. The epidemiology of salmonella infection of calves: The role of dealers. *Epidemiol. Infect.* 105(2): 295-305.
106. Wray, C., Q. C. Wadsworth, D. W. Richards and J. H. Morgan. 1989. A three-year study of *Salmonella dublin* infection in a closed dairy herd. *Vet. Rec.* 124(20): 532-535.
107. Wray, C., R. J. Callow, W. J. Sojka and P. C. Jones. 1981. A study of the antiglobulin test for the diagnosis of *Salmonella dublin* infection of cattle. *Br. Vet. J.* 137: 53-59.
108. Wray, C. and W. J. Sojka. 1976. A study of the complement fixation test in *Salmonella dublin* infection. *Res. Vet. Sci.* 21: 184-189.
109. Wray, C. and W. J. Sojka. 1977. Reviews of the progress of dairy science: Bovine salmonellosis. *J. Dairy Res.* 44(2): 383-425.

Vita

Kwankate Kanistanon

Kate was born on 21 May 1969 in Bangkok, Thailand into a family with a military tradition. She has two sisters-- older and younger. After finishing high school, she went to Khon Kaen University's School of Veterinary Medicine for 6 years and graduated in 1993. She received a scholarship from the Thai Government in 1994 to study at Virginia-Maryland Regional College of Veterinary Medicine. The scholarship is for M.S. and Ph.D., specifically in the field of Veterinary Epidemiology. Presently, she is a Master's candidate. Her next goal is to earn a Ph.D. and go back to Thailand in order to work as a faculty member at the veterinary school at Khon Kaen University.

Education

D.V.M., Khon Kaen University. Khon Kaen, Thailand.

Currently a M.S. Student, Virginia-Maryland Regional College of Veterinary Medicine. Virginia, USA.

Permanent Address

Public Health Department, College of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand. 40002.