OCCURRENCE OF *SALMONELLA* AND *LISTERIA MONOCYTOGENES* IN READY TO

EAT MEATS IN THE UNITED STATES: 2000-2010

A Paper
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Anthony Wilfred Ntaate

In Partial Fulfilment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Program:
Food Safety

May 2012

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Occurrence of *Salmonella* and *Listeria Monocytogenes* in ready to eat

meats in the United States: 2000-2010

By

Anthony Wilfred Ntaate

The Supervisory Committee certifies that this *disquisition* complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Margaret Khaitsa
Chair

Charlene Wolf Hall

Neil Dyer

John McEvoy

Approved:

6/19/2012   Deland Myers

Date   Department Chair

ABSTRACT

The purpose of this review is to characterize the occurrence of *Salmonella* and *Listeria monocytogenes* in ready to eat (RTE) meats in the United States between the years 2000-2010. Data were obtained from the CDC foodborne outbreak online database, morbidity and mortality weekly reports, summary of notifiable diseases and the foodborne outbreaks page. Additional information was obtained from peer reviewed journals. RTE roast pork, turkey deli meat, and Italian type salami meats were the vehicles in the *Salmonella* outbreaks reported. Half of the eight outbreaks reviewed were multistate in nature affecting many states and the rest were sporadic. The *Salmonella* serotypes isolated were *Salmonella* Uganda, *Salmonella* Hadar, *Salmonella* Montevideo, whereas the *L. monocytogenes* serotypes *were* 1/2a and 4b. The major risk factors for listeriosis and salmonellosis outbreaks were being elderly and having an underlying immunocompromising medical condition. Pregnant women were particularly at risk for listeriosis.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

More than 200 known diseases are transmitted through food as the vehicle and the causes

of foodborne illness include viruses, bacteria, parasites, toxins, metals, and prions*(43)*.

According to a 2011 report by Scallan et al, foodborne diseases cause an estimated 48 million

illnesses with 128,000 hospitalizations and 3000 deaths annually in the United States of America

(US)*(54, 55)*. A total of 31 pathogens cause approximately 37.2 million foodborne illnesses each

year in the US, of which 5.5 million (59%) are caused by viruses, 3.6 million (39%) by bacteria,

and 0.2 million (2%) by parasites *(47)*. Among those outbreaks with a laboratory confirmed

single etiologic agent, *Salmonella* causes 35%, and 28% of foodborne disease related

hospitalizations and deaths, respectively while *Listeria monocytogenes* has been linked to 19%

of foodborne disease related deaths in the US *(54)*.

According to the Foodborne Disease Active Surveillance Network (FoodNet)/CDC

annual surveillance reports, the incidence of salmonellosis and listeriosis per 100,000 people in

the US increased from 14.2 in 2000 to 17.6 in 2010 for salmonellosis while no such increase

seen with listeriosis at 0.34 during the same period *(9)*. It is important to note that these values

are above the national healthy people 2010 objective of 6.8 and 0.25 for salmonellosis and

listeriosis, respectively *(9)*. The safety concern of *Salmonella* and *L. monocytogenes* in RTE

meats is highlighted by several well publicized outbreaks *(22, 56, and 37)*. In addition, the recall

of 26 million pounds of RTE turkey meats in 2002 underscores the economic consequences of

RTE meats contamination with *Salmonella* or *L. monocytogenes (31, 45)*.In 1989, the US

Department of Agriculture (USDA) established a link between human listeriosis and a plant that

produced turkey frankfurters and instituted a zero tolerance policy for *L. monocytogenes* in RTE

meat products *(64, 58),* which was later to include *Salmonella (44)*. In 1996, the USDA/FSIS in

1

order to fill the gap in the regulation and inspection system, and the lack of adequate measures to address the problem of pathogenic microorganisms on meat and poultry products, introduced the pathogen reduction/Hazard Analysis and Critical Control Point System (HACCP) requiring meat processing firms to have HACCP systems to meet the *Salmonella* pathogen reduction performance standards set forth in 9 CFR part 310. 25 (b) and 9 CFR part 381.94 (b), and the *L. monocytogenes* pathogen reduction standards set forth in 9 CFR part 430 *(59);* therefore it became very important to prevent the contamination of RTE meats with these pathogens.

The purpose of this review paper was to describe the occurrence of *Salmonella* and *L. monocytogenes* in RTE meats in the US in respect to spatial and temporal trends, serotypes, vehicles of transmission, risk factors and diagnostic methods for the period between the years 2000 to 2010.

OBJECTIVES

The objectives of this review paper were to document the occurrence of *Salmonella* and

L. monocytogenes in RTE meats in the U.S between the years 2000 to 2010 by,

    I.   Vehicles of transmission

    II.   Associated risk factors

    III.   Serotypes involved

    IV.   Spatial and temporal trends

    V.   Food testing methods used

METHODOLOGY

Information on outbreaks was obtained from the CDC's Foodborne Outbreak Online Database (FOOD) available at; http://wwwn.cdc.gov/foodborneoutbreaks/, the CDC Morbidity and Mortality Weekly Reports, available at; http://www.cdc.gov/mmwr/mmwr_wk/wk_pvol.html, the CDC Summary of notifiable Diseases 2000-2010, available at: http://www.cdc.gov/mmwr/mmwr_nd/, and the Foodborne outbreaks page, available at CDC's foodborne outbreak response team page, available at: http://www.cdc.gov/outbreaknet/outbreaks.html. Additional information was obtained by searching peer reviewed journals available online by using search engines PubMed and ScienceDirect.

LITERATURE REVIEW

Salmonella and salmonellosis

Salmonella is a Gram negative, rod shaped, motile bacterium which occurs widely in nature including soil and water, and animals especially swine and poultry (*18, 26*). *Salmonella* is divided into two species *S. enterica* and *S. bongori*, with *S. enterica* further subdivided into six sub species which include *S. enterica* subsp. *enterica*,*S. enterica* subsp. *salamae*, *S.enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae, S enterica* subsp. *houtenae* and *S enterica* subsp. *indica (15).* The majority of human pathogenic serotypes belong to *S. enterica* sub sp.*enterica,*which has been described to contain more than 2000 serotypes *(18, 10). S. enterica* sub sp.*enterica*serotypes are usually isolated from humans and warm blooded animals and the other sub species from cold bloodied animals and the environment *(18). S. enterica* sub sp.*enterica* serotypes may be host specific; host restricted, or can occur in a broad range of animals. For example *S.* Typhi; *S.* Gallinarum are specific to humans and poultry respectively, *S.* Dublin is restricted to ruminants though it can be isolated in other animals whereas *S.* Typhimurium and *S.* Enteritidis cause disease in a broad range of animals *(18).* Salmonellosis is the condition caused by infection with *Salmonella* and it manifests as diarrhea, fever and abdominal cramps, and can establish as a localized infection or enter the blood stream *(8).* The disease affects all age groups although infants, immune compromised and the elderly are at great risk of developing severe disease *(8).*According to a 2010 CDC report, non typhoidal salmonellosis caused the highest number of hospitalizations (35%) and death (28%) compared to other pathogens that caused foodborne illness that year *(55).*

<center>*Listeria* and listeriosis</center>

The bacterial genus *Listeria* contains six species;*L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri, L. seeligeri*, and L.grayi. *Listeria* is a motile, rod shaped, Gram positive bacteria *(53)*.The temperature limits for *Listeria* growth are 32^oF-113^oF, with optimal growth at pH7, though it thrives between pH 4.5 and pH 9.2*(52).Listeria* species are generally ubiquitous and can be isolated in different environments including water, soil vegetation, sewage, farm and food processing areas *(53)*. Using serological techniques that detect interactions between *Listeria* somatic (O)/flagellar (H) antigens and their corresponding antisera, *Listeria* can be subdivided into 15 serotypes;*L. monocytogenes* consists of serotypes 1/2a, 1/2b, 1/2c, 3a, 3b, 3c,4a, 4ab, 4b, 4c, 4d, 4e, and 7; *L. ivanovii* serotype 5; *L. innocua* serotypes 4ab, 6a, and 6b; *L. welshimeri* serotypes 6a and 6b; *L.grayi* serotype Grayi and *L. seelegeri* of serotypes 1/2b, 4c, 4d, and 6b *(19).* It is important to note that over 98% of isolations from clinical cases of human listeriosis belong to *L. monocytogenes* serotypes 4b, 1/2a, 1/2b, 1/2c with serotype 4b accounting for 49% of all the cases *(19).*Among all species in the genus *Listeria*, only *L. monocytogenes* is typically implicated in human foodborne illness*(60).*

Listeriosis tends to be more severe in immunocompromised individuals such as pregnant women, neonates, the elderly, and individuals under chemo therapy that have suppressed immune functions *(19).*In the elderly and immunocompromised, sepsis and meningitis are the main clinical presentations. Pregnant women may experience a mild, flu-like illness followed by fetal loss or bacteremia and meningitis in the newborns. Meanwhile, immunocompetent individuals may experience acute febrile gastroenteritis *(12).*

<center>6</center>

Foodborne illness surveillance in the United States

Food is certainly not sterile, nor can eating be guaranteed risk free; however, an informed consumer is the first step in the process of food safety *(43)*. Foodborne disease is caused by consuming contaminated foods *(43)*. Although more than 250 different foodborne diseases have been described, most of these diseases are infections, caused by a variety of bacteria, viruses, and parasites *(43)*. Others are poisonings caused by harmful toxins or chemicals that have contaminated the food. The infections are diagnosed by specific laboratory tests that identify the causative organism. Developing and maintaining an adequate public health infrastructure capable of conducting surveillance of foodborne diseases as well as their epidemiologic investigation is critical *(16)*. Also the coordination of local, state, and national organizations is necessary given the evolving nature of these outbreaks *(16)*.

Nationally in the US, FoodNet; a collaborative project among CDC, ten State health departments(Connecticut, Georgia, Maryland, Minnesota, New Mexico, Oregon, Tennessee, California, Colorado and Newyork), the Food Safety and Inspection Service (FSIS) of the USDA, the Center for Food Safety and Applied Nutrition (CFSAN), and the Center for Veterinary Medicine (CVM) of the US Food and Drug Administration (FDA) is responsible for conducting surveillance to produce stable and accurate national estimates of the burden and sources of foodborne diseases *(9)*. This enhanced surveillance and investigation conducted by FoodNet is integral in developing and evaluating new prevention and control strategies to improve food safety and public health. Since January 1996, the foodborne disease active surveillance network has been collecting population based active surveillance data on both *Salmonella* and *L. monocytogenes (9)*. Unlike salmonellosis which has been a notifiable disease

condition since 1992, listeriosis was added to the list of nationally notifiable diseases in the US in 2001 *(13, 15)*.

PulseNet is a national network of local, State, territorial, agricultural, and federal laboratories in all 50 states and 82 countries coordinated by CDC and the Association of Public Health Laboratories, that subtypes and categorizes foodborne bacterial pathogens through a standardized process of pulsed field gel electrophoresis (PFGE). PulseNet allows investigators from participating sites to upload PFGE patterns to an electronic database and compare them with patterns of other pathogens isolated from humans, animals, and foods to identify matches and possible linkages between pathogens especially during outbreaks *(11)*.

In notifiable disease surveillance, health care providers and laboratory professionals are required by law to report individual cases of disease when selected pathogens are identified in patient specimens or specific clinical syndromes are recognized. Local public health agencies report these diseases to the state or territorial public health agency, which in turn voluntarily submits the information to the National Notifiable Diseases Surveillance System, which CDC oversees *(11)*.

CDC also collects reports of foodborne outbreaks due to enteric bacterial, viral, parasitic, and chemical agents. State, local, and territorial public health agencies report these outbreaks to the Foodborne Disease Outbreak Surveillance System through the National Outbreak Reporting System (NORS). The CDC surveillance team conducts analyses of these data to improve understanding of the human health impact of foodborne outbreaks and the pathogens, foods, settings, and contributing factors *(11)*.

Control of *Salmonella* and *Listeria monocytogenes* in ready to eat meats in the United States

The safety concern of *Salmonella* and *L. monocytogenes* in RTE meats is highlighted by several well publicized outbreaks involving RTE meat products. In the year 2000 for example, an outbreak of listeriosis involving deli turkey meat resulted in 30 illnesses, 4 deaths, and 3 miscarriages or stillbirths *(56).* In 2010, a multistate outbreak of 272 cases of *Salmonella Montevideo* infections in 44 states and the District of Columbia that occurred between July 2009 and April 2010 andwere associated with consumption of RTE Italian style salami meats, resulted in hospitalization of 52 patients though no death was recorded *(37).* The recall of 26 million pounds of RTE turkey meats contaminated with *L. monocytogenes*in 2002 underscores the economic consequences of RTE meats contamination with *Salmonella* or *L. monocytogenes (31, 45).*USDA/FSIS has been conducting a regulatory microbiological testing program on RTE meat and poultry products from processing establishments since 1983 in order to control microbial hazards of public health concern including *Salmonella* and *L. monocytogenes(62).*In 1989, the USDA established a zero tolerance policy for *L.monocytogenes* in RTE meat products *(58),* which was later to include *Salmonella(44).* This decision was taken because of a link, established in 1989 between human listeriosis and a plant that produced turkey frankfurters *(64).* In 1996, the USDA/FSIS in order to fill the gap in the regulation and inspection system, and the lack of adequate measures to address the problem of pathogenic microorganisms on meat and poultry products, introduced the pathogen reduction/Hazard Analyisis and Critical Control System (HACCP) requiring meat processing firms to have HACCP systems to meet the *Salmonella* pathogen reduction performance standards set forth in 9 CFR 310. 25 (b) and 9 CFR 381.94 (b), and the *L. monocytogenes* pathogen reduction standards set forth in 9 CFR 430 *(59),* Therefore it became very important to prevent the contamination of RTE meats with these pathogens.

The U.S. Department of Agriculture's Food Safety and InspectionService (FSIS), in 2003 issued an interim final rule requiring federal establishments producing RTE meat and poultry products take meaningful steps to further reduce the incidence of *Listeria monocytogenes (29).* All establishments that produce RTE products that are exposed to the environment after lethality treatments were required to develop written programs, such as Hazard Analysis and Critical Control Point (HACCP) systems, Sanitation Standard Operating Procedures (SSOPs) or other prerequisite programs, to control *L. monocytogenes (25).* Establishments were required to verify the effectiveness of these actions through testing and sharing the results with FSIS *(59).* FSIS were to conduct its own verification activities for each establishment's *Listeria* control program. The level of verification was to depend on the *L. monocytogenes* control strategy for any given establishment. According to FSIS the alternatives that establishments were to select from were as follows *(59):*

I.   Alternative 1-Employ both a post-lethality treatment and a growth inhibitor for *Listeria* on RTE products. Establishments opting for this alternative were to be subject to FSIS verification activity that focuses on the post-lethality treatment effectiveness.

II. Alternative 2-Employ either a post-lethality treatment or a growth inhibitor for *Listeria* on RTE products. Establishments opting for this alternative were to be subject to more frequent FSIS verification activity than for Alternative I.

III. Alternative 3-Employ sanitation measures only. Establishments opting for this alternative were to be targeted with the most frequent level of FSIS verification activity. Within this alternative, FSIS placed increased scrutiny on operations that produced hotdogs and deli meats because in a 2001 risk ranking, FSIS and the Food and Drug

Administration identified these products as posing relative high-risk for illness and death.

The Food Code which the US Food and Drug Administration publishes, serves as a model that assists food control jurisdictions at all levels of government including local, State, tribal, and federal regulators by providing them with a scientifically sound technical and legal basis for regulating the retail and food service segment of the industry (restaurants and grocery stores and institutions such as nursing homes). Local authorities in states update their own food safety rules and to be consistent with national food regulatory policy outlined in the food code *(27)*. There are clear guidelines in chapter 3 of the FDA Food Code for grocery stores on how to handle ready to eat or other potentially hazardous food. Manufacturers of RTE meats are required to indicate the date/day by which the food shall be sold, or discarded when held at a temperature of $5^{o}C$ ($41^{o}F$) or less for a maximum of 7 days *(27)*.

Common testing methods for *Salmonella* and *Listeria monocytogenes* in RTE meats in the United States

Culture methods for isolation and detection of *Salmonella* and *Listeria monocytogenes* in RTE meats

For detection of *Salmonella* in the US, the FDA recommended a culture method which has been described in the bacteriological analytic manual *(2)*. The pre-enrichment procedure involves aseptically weighing 25 g of RTE meat sample and adding 225mls of sterile lactose broth, followed by homogenization at room temperature and pH 6.8. The sample mixture is then incubated for 24 hours (h) at $35^{o}C$. Selective enrichment involves transfer 0.1ml of sample to 10 mls of Rappaport-Vassiliadis (RV) medium and another 1 ml mixture to 10 ml of Tetrathionate

(TT) broth. The RV medium and TT broth are both incubated for 24 h at 42°C and 43°C

respectively. Differential plating is done by streaking 3 mm loopful (10μl) of incubated TT broth

on bismuthsulfite (BS) agar, xylose lysine desoxycholate (XLD) agar and hektoen enteric (HE)

agar. This is repeated for the RV medium and incubated for 24 h. Plates are examined for

presence of colonies that may be of *Salmonella*. Typical *Salmonella* colonies appear a) blue-

green to blue on HE agar, b) pink colonies with or without black center on XLD agar, and c)

brown/gray/black on BS agar. If colonies are present further plating is carried out by picking 2 or

more and incubating in Triple Sugar Iron (TSI) and Lysine Iron Agar (LIA) slants. The TSI and

LIA slants are incubated at 35°C for 24 hours. *Salmonella* typically produces alkaline purple

reaction in the tube. *Salmonella* cultures also produce hydrogen sulphide (H2S). Confirmation is

done by applying biochemical and serological identification tests to *Salmonella* positive TSI and

LIA samples. Other similar methods have been described by the Association of Official Analytic

Chemists (AOAC) and the USDA/FSIS *(34, 61).* The USDA method is different only because

Buffered Peptone Water (BPW) is used instead of lactose broth during pre-enrichment.

Additionally Brilliant Green Gulfa (BGS) and Xylosine Lysine Tergitol (XLT4) agars the ones

used for differential plating, however there use has been showed not to have any specific

advantages compared to the other agars *(2).* These methods were evaluated and found to have a

detection limit of approximately < 0.01 MPN per 25 g of RTE meat sample *(4).* Although the

specificity of these methods is high, the sensitivity can at times be low, due to a high number of

false negatives that can occur as a result of difficulties in recovering sub lethally injured bacterial

cells. These methods are generally labor intensive and require a minimum of 5-7 days for

complete analysis hence the need for the development of faster independent screening and

detection methods for this pathogen in foods. As for *L. monocytogenes*, the FDA recommended a

method that is as described in chapter 10 of the bacteriological analytic manual *(3).* The pre-enrichment step involves aseptically weighing 25g of RTE meat sample and adding 225ml of Buffered *Listeria* Enrichment Broth (BLEB), at 30° C for 4 h and at the fourth hour of incubation, the selective agents (acriflavin, 10 mg/L (4, 40); sodium nalidixate, 40 mg/L; optional antifungal, e.g. cycloheximide 50 mg/L) are added. Incubation is continued at 30° C for a total of 48 h. Selective enrichment culture is streaked at 24 and 48 h on one of the prescribed differential selective-agars in order to isolate *Listeria* species (Oxford agar, PALCAM, LPM plus esculin and ferric iron, MOX). Oxford agar is still the preferred standard selective isolation medium. Incubate at 35° C for 24-48 h; *Listeria* colonies are black with a black halo on these esculin-containing media. Certain other bacteria can form weakly brownish black colonies, but color development takes longer than 2 days. Five or more typical colonies are transferred from OXA and PALCAM or modified LPM or MOX to Trypticase soy agar with yeast extract (TSAye), and incubated at 30° C for 24-48 h. Blue colonies are presumptive *L. monocytogenes* colonies since L. ivanovii is not often reported in foods. Confirmation for *L. monocytogenes* is done using Biochemical and serological tests. Some of the rapid kits available to confirm *L. monocytogenes* include: AccuProbeTM *Listeria monocytogenes* culture confirmation test (Gen-Probe, Inc, San Diego, CA; 10, 19). GeneTrak Listeria monocytogenes test kit (Neogen, Lansing, MI; 19). Probelia *Listeria monocytogenes* test kit (BioControl, Seattle, WA).VIDAS *Listeria monocytogenes* test kit (bioMerieux). Transia Plate *Listeria monocytogenes* (Diffchamb SA, Lyon, France). The limitations of these traditional culture methods are similar to those described for *Salmonella*. The USDA/FSIS has a slightly modified method which is described in its microbiological guide book *(60);* this standard culture method is similar to the AOAC culture method *(34).* For quality control purposes, this protocol requires that for each set of concurrently

13

analyzed samples, there must be an *L. monocytogenes* positive and one un-inoculated media (negative) controls. The pre-enrichment step involves aseptically weighing 25 g of RTE meat sample and adding 225 ml of Modified University of Vermont (UVM1) broth followed by pummelling in a stomacher for 2 min, the mixture is incubated at 30°C for 22 h. These is followed by transfer 0.1 ml of UVM1 enrichment broth to 10 ml Morpholine propanesulfonic acid-buffered Listeria enrichment broth (MOPS-BLEB) or 10ml Fraser broth (FB) and incubated 35°C for 26 h. Selective enrichment is achieved by streaking 0.1 ml from UVM1 enrichment; FB; or MOPS-BLEB broths on to Modified Oxford agar (MOX) plates and incubating at 35°C for 26 h. MOX plates are examined for colonies with morphology typical of *Listeria* spp (small surrounded by a zone of darkening due to esculin hydrolysis). Secondary selective enrichment is carried out by sweeping that many colonies present on the MOX agar on to Horse blood overlay agar (HL) and incubating at 35°C for 22 h. Examination is done against backlight for translucent colonies surrounded by a small zone of â-hemolysis. Confirmatory testing is done by taking at least one suspect clearly isolated colony using biochemical, CAMP factor test, and genetic identification tests.

Biochemical and serological identification of *Salmonella* and *Listeria monocytogenes*

After Isolation, suspect bacteria are identified as *Salmonella* if they are Gram negative, rod shaped, facultatively anaerobic, catalase positive, oxidase negative, urease negative and non-spore forming *(20)*. Biochemical confirmation can be carried out using a number of tests which include Triple sugar iron agar; Lysiene iron agar; Urease test (*Salmonella* is urease negative); Phenol red dulcitol broth (Positive test indicated by gas formation, and acid reaction which appears yellow), ornithine decarboxylase, and Lysine decarboxylase tests *(20, 34)*. Serological tests can be carried by determining the antigenic composition. The antigens are classified as

somantic (O) or flagella (H) and are determined by agglutination using somatic and flagella poly valent antisera. These conventional biochemical tests are time consuming hence miniaturized commercial testing kits have been developed such as the 20E (bioMerieux, Hazelwood, MO) and BBL^{TM} Enterotube^{TM} (BD diagnostics, Sparks, MD), which use sugar utilization and provides an efficient way of identifying suspected *Salmonella* isolates (20). *L. monocytogenes* on the other hand are Gram positive, non-spore forming, catalase positive, indole and oxidase negative, aerobic or facultatively anaerobic *(19). L. monocytogenes* is also hemolytic on horse or sheep blood agar.The Christie, Atkins, Munch-Peterson (CAMP) test is also used to differentiate *Listeria* species and is carried out by streaking a â-hemolysin-producing *Staphylococcus aureus* strain and *Rhodococcus equi* parallel to each other on sheep blood agar plate. Suspect cultures are streaked at right angles in between (but not touching) the two streaks. Hemolysis by *L. monocytogenes* and to a lesser degree *L. seeligeri* is enhanced in the vicinity of *S. aureus* and hemolysis by *L. ivanovii* is enhanced in the vicinity of the *R. equi* streak *(3).* Agglutination and enzyme based immunoassay employing poly clonal and monoclonal antibodies to *Listeria*, are examples of serological methods for detection. A number of commercial kits on the basis serological reactivity are available commercially such as, Assurance *Listeria* EIA, EIA Foss *Listeria*, Lister Test *Listeria* Rapid Test and TECRA *Listeria* Visual Immuno Assay among others *(19).* ELISA methods that use an antibody immobilized to a microtitre well for antigen capture in combination with a secondary antibody coupled to an enzyme to detect the captured antigen, are widely applied because they can be used with difficult sample matrices which makes these tests particularly well suited for food testing. The inability of these serotyping methods to correlate serovars directly with species, limits their use, the high cost of acquiring specific sub

15

type antisera, and their ability to give occasional discrepant results limits their use in food testing.

Molecular based methods for detection of *Salmonella* and *Listeria monocytogenes*

Molecular based methods detect DNA or RNA specific for a target organism and are becoming increasingly popular in food microbiology because of their sensitivity, specificity and accuracy in identifying *Salmonella and L. monocytogenes*. Commonly used methods include DNA hybridization which detects a target DNA sequence using a complimentary oligonucleotide probe with a label for detection. Previously radioactive isotopes incorporated into oligonucleotide sequence were used as labels for detection. More recently, biotinylated probes or fluorescent markers allow detection of target sequences with equivalent sensitivities to radioactive probes *(34)*. Traditional polymerase chain reaction (PCR) is another molecular technique commonly used in the US to detect *Salmonella* and *L. monocytogenes*. This method leads to amplification of a specific DNA fragment in a process that involves denaturing of target DNA at high temperature, annealing two synthetic oligonucleotides (primers) to opposite strands at a temperature that allows hybridization to correct target, and polymerization with oligonucleotides as primers for the enzymes and the target DNA as template *(49)*. Traditional PCR is however affected by two draw backs that include end point quantification and opening the reaction tube to verify reaction product specificity can lead to contamination. To address these problems, a plethora of novel real time and reverse transcription PCR methods have been developed for rapid detection of *Salmonella* and *Listeria monocytones(35, 41, 65)*. Real time PCR allows quantification of initial target copy numbers and evaluation of product specificity without opening the reaction chamber hence saving time and reducing contamination risk *(21)*. Assays are designed to target short DNA fragments using primers specifically selected to avoid

primer dimers. There is an increase in fluorescence in response to amplicon formation during thermo cycling and this is achieved by use of double stranded DNA binding dyes, or sequence specific probes that generate a signal in the presence of the target DNA. The principle is that the earlier the increase in fluorescence the larger the initial target copy number present in the reaction *(21, 28).* Reverse Transcription PCR (RT-PCR), involves a two-step reaction, with the first involving a reverse transcriptase enzyme translating mRNA an indicator of cell viability into complementary DNA. The reaction is usually initiated by random oligonucleotide primers. In the second step the complimentary DNA is used as template for amplification of specific sequences by PCR using target specific oligonucleotide primers and a DNA polymerase to facilitate the reaction. RT- PCR is used for detection of viable foodborne pathogens *(21).* The disadvantage is that it is time consuming. During the review period, Many PCR assays have been developed for the rapid detection of *Salmonella* and *L. monocytogenes*. In 2003, Ellingson et al developed a 12 hour real time PCR technique (after pre/selective enrichment) for the sensitive and specific detection of *Salmonella* in raw and RTE meat products *(23),* likewise Almeida et al, in 2000 was also able to use *inl*A gene sequences as a target to detect pathogenic *L. monocytogenes (1).* The use of PCR methods is dramatically affected by presence of inhibitory/contaminating substances in foods. Traditional and real time PCR methods cannot distinguish between live and dead cells hence have a potential of providing unreliable results. Conventional PCR methods have a high sensitivity with a detection of limit of 10^3CFU/ ml *(49).* Their specificity can at times be low due to the possibility of high false positives arising as a result of detecting DNA of non-viable bacteria. To improve on the sensitivity, it is important to carry out selective enrichment of samples in order to raise the number of non-viable bacteria *(5).* It is important to note that a number of new molecular detection methods have been described like nucleic acid sequence

based amplification, DNA microarrays, and oligonucleotide based microarrays, but they are not

yet commonly used in the US to detect *Salmonella* and *L. monocytogenes* from RTE meat

samples.

Molecular sub typing and pulsed field gel electrophoresis of *Salmonella* and *Listeria*

monocytogenes

Many molecular typing techniques have been developed for purposes of finger printing

bacteria. These techniques involve use of restriction enzymes that recognize and cut particular

sequences with in the DNA molecule. Genetic relatedness is then determined by comparison of

the number and size of fragments which are separated and visualized using electrophoresis.

Although there is a wide array of DNA fingerprinting methods used for the purpose of subtyping

bacteria, restriction fragment length polymorphism (RFLP) is one of the most frequently used

molecular subtyping tools in epidemiologic investigations. While there are different approaches

to RFLP like multilocus enzyme electrophoresis and ribotyping, in the US pulsed field gel

electrophoresis (PFGE), has been shown to be a reliable and highly discriminating method for

subtyping foodborne pathogens and other bacteria *(19).* PFGE entails the digestion of genomic

DNA by one or more restriction enzymes *XbaI; BlnI*; and *SpeI,* separation of the restriction

fragments by field inversion of electrophoresis, and resolution of fragments in agarose gels *(35,*

52). Its ability to differentiate among epidemiologically unrelated isolates compared to the other

methods allows it to be used for source tracking; however it is not automated and hence labor

intensive *(35)*. PFGE is the method used by laboratories in PulseNet, a network of state and local

health departments, and other public health laboratories in the US to fingerprint *Salmonella* and

L. monocytogenes and other bacteria *(52)*. Before performing PFGE, sample DNA from

Salmonella is prepared through the following steps; *Salmonella* is grown on Trypticase soy agar

plates with 5% sheep blood (TSA-SB; Becton Dickinson and Company, Sparks, MD) at 37°C for 14–16 h. All isolates are identified and serotyped using standard procedure, Cell suspensions are prepared by removing cells from the plate surface with a sterile cotton or polyester fiber applicator swab that has been moistened with sterile Cell Suspension Buffer (CSB, 100 mM Tris, 100 mM EDTA [pH 8.0]) and transfer them to tubes (Falcon 2057, 12 75 mm; Becton Dickinson, Franklin Lakes, NJ) containing 2 mL of CSB. The concentration of each cell suspension is adjusted to a turbidity reading of 0.48–0.52 on the digital output of a Microscan Turbidity Meter (Dade Behring, Inc., Deerfield, IL). This corresponds to absorbance values of approximately 1.3–1.4 measured at a wavelength of 610 nm with a spectrophotometer (Shimadzu Corp., Kyoto, Japan) and transmittance values of approximately 15% when using a Vitek colorimeter (bioMérieux, Durham, NC). A 400µL aliquot of each adjusted cell suspension is transferred to a sterile microcentrifugeb tube containing 20µL of proteinase K(20 mg/mL stock; Amresco, Solon, OH; Invitrogen, Carlsbad, CA) and mixed gently by tapping a capped tube ping a capped tube on the palm of the hand or flicking it several times with fingers. Alternatively,the proteinase K can be added directly to each cell suspension after they have been aliquoted into their respective tubes. The agarose used to make the plugs consists of 1% SeaKem Gold agarose (SKG, Cambrex, and Rockland, ME) and 1% sodium dodecyl sulfate (SDS; Roche Diagnostics Corp., Indianapolis, IN) prepared in Tris EDTA buffer (TE; 10 mMn Tris, 1 mM EDTA [pH 8.0]). Ribot et al in 2006 described the protocol used by PulseNet to subtype *Salmonella (52),* and is reproduced in this review (see appendix 5). Graves et al in 2001 described the standardized protocol used by PulseNet to subtype *L. monocytogenes (52).*

Alternatively DNA from *L. monocytogenes* is prepared as follows; Bacteria is grown on brain heart infusion agar plates at 37°C for 16–18 h. Cells are removed from the plate to plastic

tubes (Falcon 2057, 14 ml-17×100 mm) containing 3 ml of TE buffer using a sterile cotton swab and the cell density adjusted. The standardized cell suspension (240 μl) is transferred to 1.5 ml microcentrifuge tubes. Sixty microliters of 10 mg/ml lysozyme solution (Sigma, St. Louis, MO) is added and mixed with the cells by pipetting up and down. The mixture is incubated in a water bath at 37°C for 10 min. An equal volume of molten 1.2% SeaKem Gold agarose, 1% sodium dodecyl sulfate, 0.2 mg/ml Proteinase K (Roche Molecular Biochemicals, Indianapolis, IN) (SSP) prepared in sterile distilled water and maintained at 53–56°C is added to the cell suspension and mixed by gently pipetting up and down several times. The mixture (600 μl) is dispensed into two forms (300 μl each) of a sample reusable plug mold (Bio-Rad, Hercules, CA) and allowed to cool for 5 min. The agarose plugs are transferred to 50 ml polypropylene conical tubes (Becton Dickinson, Franklin Lakes, NJ) containing 4 ml of lysis buffer (50 mM Tris pH 8.0, 50 mM EDTA, pH 8.0, 1% sodium lauryl sarcosine, 0.15 mg/ml Proteinase K), incubated for 2 h at 50–54°C in an orbital water bath shaker (Lab-Line, Melrose Park, IL) and shake at 200 rpm. After proteolysis, the lysis buffer solution is removed and the plugs are washed twice with 15 ml of preheated (50–54°C) sterile distilled water for 10 min each followed by four washes with 15 ml of preheated (50–54°C) TE buffer for 15 min each in the orbital water bath shaker (50–54°C) at 200 rpm. After the final TE wash, the plugs are sliced (2–2.5 mm slices) using a Gel-Cutting Fixture (S&S Service Company, Stockbridge, GA) and prepared for restriction digestion or stored in 1.5 ml TE at 4°C until ready for restriction.

Foodborne outbreaks associated with *Salmonella* and *Listeria monocytogenes* on RTE meats in

US; 2000-2010

Outbreaks associated with *Salmonella*

Three outbreaks have been documented to have occurred between 2000-2010 involving

these RTE meat and specifically, between July-August 2001 (Figure 1), the New York City

Department of Health and Mental Hygiene reported an outbreak of *Salmonella enterica* serotype

Uganda among 11 case patients of individuals who had eaten ready to eat roast pork,

Concurrently the Chicago Department of public health reported 12 cases of people with the same

serotype who had a history of having eaten carnitas (fried pieces of ready to eat roast pork

*(36).*Outbreak isolates were compared by molecular typing with PFGE with standardized

PulseNet protocols. A PFGE comparison showed the two outbreak strains in both cities were

distinguishable from each other. RTE roast pork was identified as the vehicle because

investigations revealed that processed meats were being stored at improper temperatures and that

potential sources of cross-contamination were not controlled in both cases *(36).* In 2008 the Iowa

Department of Public Health reported an outbreak in May of *Salmonella* Hadar infections in 3

people in the state (Figure 1). Investigations revealed sliced turkey to have been the vehicle of

spread though the food products were home prepared in all the cases *(47).*In 2010, the CDC

reported a multistate outbreak of 272 cases of *Salmonella* Montevideo infections in 44 states and

the District of Columbia (Figure 1), that occurred between July 2009 and April 2010 which were

associated with consumption of ready to eat Italian style salami meats *(37).* Diagnosis was

carried out by PulseNet, the national molecular subtyping network for foodborne disease

surveillance, and detected a cluster of *Salmonella* Montevideo infections with an

indistinguishable pulse-field gel electrophoresis (PFGE) pattern (XbaI PFGE pattern

JIXX01.0011).The infections were reported in the following states/territories; Rhode Island, Washington, Alaska, Alabama, Arizona, California, Colorado, Connecticut, District of Columbia, Delaware, Florida, Georgia, Iowa, Idaho, Illinois, Indiana, Kansas, Louisiana, Massachusetts, Maryland, Maine, Michigan, Minnesota, Missouri, Mississippi, North Carolina, North Dakota, Nebraska, New Hampshire, New Jersey, New York, Ohio, Oklahoma, Oregon, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Utah, Virginia, Wisconsin, West Virginia, and Wyoming. Italian style salamis were identified as the vehicle and black and red pepper applied to these salami products was pinpointed as having been the source of these outbreaks.

Outbreaks associated with *Listeria monocytogenes*

During the review period (2000-2010), there were only four outbreaks associated with *L. monocytogenes* on RTE meats that were reported and are recorded in the CDC foodborne outbreak on line database *(14)*.

In the year 2000 (Figure 2), an outbreak that started in May caused 30 illnesses, 29 hospitalizations and 7 deaths *(56)*. Thirty patients infected with an identical strain of *L. monocytogenes* were identified in 11 states (California, Connecticut, Georgia, Massachusetts, Michigan, New York, Ohio, Pennsylvania, Tennessee, Utah, and Wisconsin). Positive samples were identified using culturing method and all isolates had the same ribotype (DUP-1053) and serotype (1/2a) and were indistinguishable by PFGE using 2 enzymes (*Asc*I PulseNet pattern GX6A16.0014 and *Apa*I PulseNet pattern GX6A12.0017). In this particular outbreak, sliced turkey deli meat was implicated as the food vehicle that was responsible for the outbreak.

In 2001 (figure 2), an outbreak that began in June caused a total of 28 illnesses in California *(14, 22)*. These were attendees of a catered birthday party associated with delicatessen

sliced turkey meat contaminated with *L. monocytogenes* in Los Angeles California. The meat slicer or Kitchen counter was suspected to be the source of contamination though sandwich turkey deli meat was implicated as specific vehicle through which the infection spread. All serotypes were 1/2a with an indistinguishable PFGE pattern *(22).*

In 2002 (Figure 2), another multistate outbreak that caused a total of 54 illnesses and 8 deaths was reported *(14, 31, 45).* In July 46 cases had been reported and confirmed in 9 states. The *L. monocytogenes* outbreak strain isolated in patients from the states of Pennsylvania, New york, New Jersey, Massachusetts, Delaware, Maryland, Connecticut, Michigan, and Illinois had a PFGE pattern indistinguishable from two environmental isolates from the floor drains of one of Pilgrim's Pride meat processing Plants located in Franconia, Pennsylvania *(31, 45).L. monocytogenes* was detected in RTE turkey deli meat. Specifically sliceable turkey deli meat was implicated and the company recalled 27.4 million pounds of fresh and frozen turkey and chicken products *(31, 45).* Positive samples were identified by culturing and PFGE was performed for subtyping. The outbreak serotype was 4b, and ribotype DUP-1044 *(31).*

Between January-June 2010 (Figure 2), there was an outbreak of invasive listeriosis among 14 patients which were reported to the Louisiana Department of Public Health *(17).* Seven patients were hospitalized resulting in two Deaths *(17).* The outbreak was associated with consumption of hog head cheese (a meat jelly made from swine heads and feet). Isolates of *L. monocytogenes* from blood samples of patients identified the serotype as 1/2a which was indistinguishable with PFGE

Figure 1. Spatial and temporal trends in occurrence of outbreaks associated with *Salmonella* in RTE meats in the United States; 2000-2010
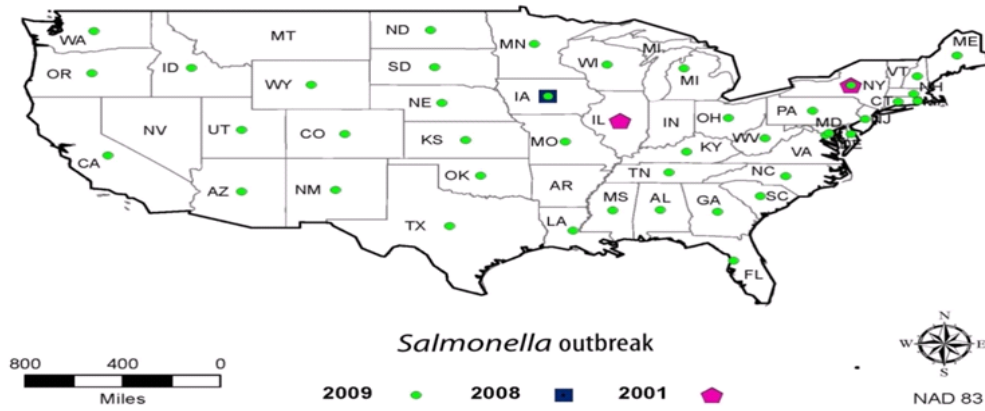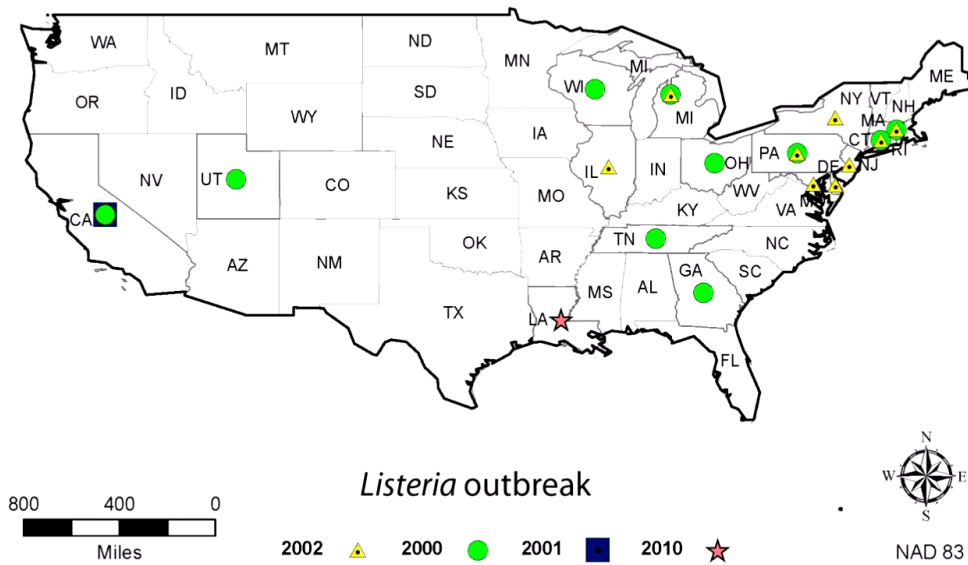

Figure 2. Spatial and temporal trends in occurrence of outbreaks associated with *Listeria monocytogenes* in RTE meats in the United States; 2000-2010

Vehicles of transmission of *Salmonella* and *Listeria monocytogenes* among the RTE meats in the

United States: 2000-2010

In 2002 Gombas et al, conducted a study to develop data on the risk of listeriosis to support a science based strategy for addressing *L. monocytogenes* in foods in the US*(30).*This was due in part to the fact that a risk assessment study conducted by the FDA, USDA, and the CDC in 1999 reported that though there were many data on *L. monocytogenes* prevalence in many RTE foods, studies with any detectable levels were rendered unacceptable because of the zero prevalence policy hence there were no benefits to monitoring levels of the pathogen in food products. In this particular study samples of RTE meats from retail markets at FoodNet sites from Nothern California and Maryland were analysed. Samples included luncheon meats, deli salads, fresh soft ripened cheeses, bagged salads, blue veined and soft mold ripened cheeses, smoked seafood, and sea food salads. Results from this study indicated a high rate of positive samples for sea food salad (4.7%) and smoked fish (4.3%). The lowest prevalence was for fresh soft cheese (0.17%) and bagged salads (0.74%), whereas the percentages of *L. monocytogenes* positive samples of delisalads and sliced luncheon meats were 2.4% and 0.89%, respectively. This study also reported a trend where store packaged deli salads, luncheon meats, and sea food salads had a higher frequency of *L. monocytogenes* than manufacturer packaged products. From the 2000 outbreak of listeriosis, sliced turkey deli meat was implicated as the food vehicle that was responsible for the outbreak *(56).* In the 2001 outbreak, sandwich turkey deli meat used to make the submarine sandwiches was implicated as specific vehicle through which the infection spread *(22),* and in the 2002 outbreak, again specifically sliceable turkey deli meat was implicated and the company recalled 27.4 million pounds of fresh and frozen turkey and chicken products *(31, 45).*In the 2010 outbreak, Hog head cheese were the specific vehicle implicated

In 2006 a study conducted by Khaitsa et al on recovery of *Salmonella* from RTE turkey meats from retail stores in the Mid-Western US indicated that recovery of the pathogen from roast turkey meats was significantly higher than other RTE meats which included drum steaks, oven roasted breast, ham, bacon and breakfast sausage *(38)*.In the 2001outbreak, ready to eat roast pork was identified as the vehicle *(36)*. In the 2008 outbreak in Iowa, sliced turkey was identified as the vehicle in the report by the Iowa Department of Public Health *(47)*. And finally in the large multistate outbreak of 2009-2010, Italian style salamis were identified as the vehicle, and black and red pepper applied to these salami products was traced back to have been the source of this outbreak.

Risk factors for *Salmonella* and *Listeria monocytogenes* 2000-2010

Studies by Scallan et al on estimates of foodborne illness in the US in 2011 *(54, 55)*and a similar previous study by Mead et al in 1999*(43),* reportedsalmonellosis and listeriosis as having a high morbidity and case fatality rates respectively, whichmakes human studies on risk factors unethical; besides the two diseases are notifiable in the US. During the 2001 outbreak of salmonellosis that has been described, it was observed that all the 23 case patients were adults of Hispanic ethnicity and eating ready to eat pork was a risk factor *(34)*. In the 2009-2010 outbreaks, the median age of case patients was 37 years (range, 1-93), 53% (144/272) were females, 26% of patients were hospitalized and no death was reported overall *(37)*.

From the listeriosis outbreak that occurred in the year 2000 where, 30 cases with identical strains were identified, eight (27%) were pregnant women or mother infant pairs, twenty two (73%) were men and non-pregnant women with a median age of 66 years. The infections resulted in the death of 4 persons (57%) aged between 67-78 years *(48)*. From the 2001 outbreak of febrile gastro enteritis, all the patients who satisfied the case definition had a median age of

15.5 years with a range of 7-66 years *(22).*From the 2002 outbreak of listeriosis in the North

Eastern US; out of the 54 cases, eight (15%) were pregnant women and 4 (7%) were neonates.

From prior medical information of 41 of the 42 non maternal-neonatal cases, thirty (71%) had

the following medical conditions or factors which were considered immunocompromising or

predisposing for listeriosis: hematologic malignancy (5 patients), corticosteroid use (4 patients),

HIV infection and/or AIDS (4 patients), solid malignancy (3 patients), diabetes (3 patients), liver

disease (3 patients), inflammatory bowel disease (3 patients), organ transplantation (2 patients),

end-stage renal disease (1 patient), rickets and malnutrition (1 patient), and sickle-cell anemia (1

patient). Most elderly case patients also had an underlying immunocompromising condition; only

4 (7%) of the 54 case patients were aged 65 years and were not immunocompromised *(31).*

During the 2010 outbreak of listerioisis that was reported in Louisiana, the medium age was 64

(range, 38-93) and six (43%) of the patients had an underlying immune compromising medical

condition *(17).*

Salmonella and *Listeria monocytogenes* serotypes isolated in outbreaks in the US; 2000-2010

The outbreak serotypes of *Salmonella* associated with the RTE meats that were isolated

during the review period, included *Salmonella enterica* serotype Uganda, *Salmonella* Hadar, and

Salmonella Montevideo *(36, 47, 37).* From the outbreaks that have been described for the review

period, the serotypes of *L. monocytogenes*that were isolated from patients were *L.*

monocytogenes serotype 1/2a for the 2000 and 2001, 2010 and serotype 4b for the 2002 outbreak

(56, 22, 31, 17).

Non-outbreak related occurrences of *Salmonella* and *Listeria monocytogenes* in RTE meats

Results of a study conducted in 2006 by Bohaychuk et al*(6),* on occurrence of pathogens on RTE meats from the retail market places in Edmonton Canada did not detect presence of *Salmonella* however 3-5% of turkey breast, beef and chicken wieners contained *L. monocytogenes*, which was the rate that had also been reported for the US at that time. All the serotypes isolated from these RTE meats were 1/2a and 1/2b. The study also indicated that the problem of *L. monocytogenes* presence on RTE meats was due to post processing contamination. Another study by Levinne et al, on pathogen testing of RTE and poultry products collected at federally inspected establishments in the US between 1990-1999 showed cumulative *Salmonella* prevalence as follows among the different categories of RTE meats: jerky, 0.31%; cooked, uncured poultry products, 0.10%; large diameter cooked sausages, 0.07%; small diameter cooked sausages, 0.20%; cooked beef, roast beef, and cooked corned beef, 0.22%; salads, spreads, and pâtés, 0.05%; and sliced ham and luncheon meat, 0.22%; dry and semidry fermented sausages, 1.43%. Additionally the *L. monocytogenes* prevalence were as follows: jerky, 0.52%; cooked, uncured poultry products, 2.12%; large diameter cooked sausages, 1.31%; small diameter cooked sausages, 3.56%; cooked beef, roast beef, and cooked corned beef, 3.09%; salads, spreads, and pâtés, 3.03%; sliced ham and luncheon meat, 5.16%; semidry fermented sausages was 3.25%*(39).* A comparative risk assessment study by Endrikat et al for *L. monocytogenes* in prepackaged versus retail sliced deli meat published in 2010, sensitivity analysis, assessing the effect of the model's consumer storage time and shelf life assumptions, found that even if retail-sliced deli meats were stored for a quarter of the time prepackaged deli meats were stored, retail-sliced product is 1.7 times more likely to result in death from listeriosis *(24).* A study on *L. monocytogenes* infections from foods prepared in commercial establishments in the US by

Varma et al between the years 2000-2003 showed that most infections were sporadic in nature due contamination during food processing *(63).*Another study by Pradhan et al published in 2011 on comparison of public health impact of *L. monocytogenes* product to product and environment to product contamination of deli meats at retail market place indicated that retail level cross contamination of RTE deli meats with *L. monocytogenes* has the potential to considerably increase the risk of human listeriosis cases and deaths, and thus precise estimates of cross contamination frequency are critical for accurate risk assessments*(51).*Another study by Uesugi et al published in 2009 revealed that a combination of an antimicrobial nisin and pulsed light is effective in the reduction of *L. monocytogenes* occurrence on RTE meat products*(57).* This development is very important since listeriosis associated with RTE meats is a major concern in the US.

DISCUSSION

There are many testing methods for *Salmonella* and *L. monocytogenes* on RTE meats that are available as described in this review; however, the CDC works in partnership with other U.S. State and Local Health Departments, USDA, the U.S Food and Drug Administration and PulseNet National Surveillance Network in investigations of outbreaks and uses standard protocols approved by those organizations. Also PFGE has been shown to be a reliable and highly discriminating method for sub typing foodborne pathogens and other bacteria and is used by PulseNet to track foodborne outbreaks across the US. The review has shown that rapid methods like PCR are highly sensitive but less specific hence good for screening purposes while cultural methods are highly specific but less sensitive hence good for confirmatory purposes. The review of the diagnostic methods also indicated that it is important to carry out pre and selective enrichment of samples as direct testing using PCR gives unreliable results *(5),* another study by Myint et al in 2006 on the effect of pre-enrichment protocol on the sensitivity and specificity of PCR for the detection of naturally contaminated *Salmonella* in raw poultry compared to conventional culture indicated that selective enrichment of samples increases sensitivity of PCR for the detection of *Salmonella* at a limit of 100 colony forming units/ml *(46).*A rapid detection (10 hour) real time PCR assay was developed by Patel et al *(50),* and was found to be as efficient as the conventional USDA microbiological procedure in detecting *Salmonella* on RTE meats. Developing rapid detection methods like this with shorter pre-enrichment times and real time data monitoring capability will benefit the food industry in preventing recall of contaminated meats by stopping contaminated products from being introduced in the market place.

From the pattern of the spatial and temporal trends in the US for the review period (2000-2010), *Salmonella* and *L. monocytogenes* occurrence on RTE meats has caused multistate

30

outbreaks *(36, 37, 56),* with the exceptions being the 2008, 2001, and the 2010 sporadic outbreaks of salmonellosis and listeriosis in Iowa and California, and Louisiana respectively *(47, 22, 17).* The multistate pattern is attributed to the fact that interstate commerce allows a mass distribution of contaminated product over a large geographic area however sporadic occurrences are also very common in the US. A study on *L. monocytogenes* infections from foods prepared in commercial establishments in the US by Varma et al between the years 2000-2003 showed that most infections were sporadic in nature due contamination during food processing *(63).*

This review has shown that any RTE meat product can be a potential source of infection for *Salmonella* and *L. monocytogenes* as contamination can occur at different stages of food processing; however sliced turkey deli meat was implicated in three of the four outbreaks of listeriosis reported*(17, 22, 31, 56).* Gombas et al, in 2003 reported that the US Food and Drug Administration, the USDA and the CDC conducted a risk assessment 1999 and found that delicatessen meats had a higher risk of serious illness and death compared to other RTE meats *(30).*Another study by Levinne et al, on pathogen testing of RTE and poultry products collected at federally inspected establishments in the US between the years 1990 to 1999 that has been described showed that both *Salmonella* and *L. monocytogenes* were highly prevalent in sliced ham and luncheon deli meats and fermented sausages.The extra/additional handling of these meats post lethality/processing at retail establishments also poses a high risk of contamination as control strategies for these pathogens are not always effective *(38, 39).*

The major risk factors that were associated with outbreaks of salmonellosis and listeriosis in the review period were having an underlying immunocompromizing medical condition, and being elderly. Pregnant women were particularly at risk for listeriosis. No unique risk factor could be associated with salmonellosis patients meaning that anybody is susceptible. A study by

Yang et al, published in 2006 on consumer phase risk assessment for *L. monocytogenes* in deli meats indicated that food handling in homes increased the estimated mean mortality by 10 (6)-fold *(66)*. Of all the home food handling practices modeled, inadequate storage particularly refrigeration temperatures, provided the greatest contribution to increased risk. The impact of cross contamination in the home was considerably less and adherence to USDA/FSIS recommendations for consumer handling of RTE foods substantially reduces the risk of listeriosis *(66)*. This explains the 2001 outbreak of invasive listeriosis in California that has been described *(22)*. The source of contamination was a single package of processed turkey breast that was stored for an undetermined number of days in a delicatessen walk in refrigerator that, upon inspection, was found to have a temperature of 11°C-12°C.

The different *Salmonella* serotypes isolated from RTE meats in the review period in the US indicate that any pathogenic serotype can cause illness however for *L. monocytogenes,* only two serotypes 1/2a (from the 2000, 2001 and 2010 outbreaks) and 4b (from the 2002) have been isolated from patients who consumed contaminated RTE meat. A study by Lianou et al 2006 on the review of the incidence and transmission of *L. monocytogenes* in retail and food service establishments indicated that serotype 4b was the most common among patients in the US followed by 1/2a, these serotypes are believed to account for 80-96% of human listerioisis incidences *(25)*.

.

CONCLUSIONS

This review shows that RTE meats are not always safe and can serve as vehicles for *Salmonella* and *L. monocytogenes* associated foodborne illness. Persons at risk in the US including older adults, pregnant women, and persons with immunocompromising conditions or therapies, should take additional precautions to lower their risk for infection. This information underscores the need to have active surveillance of foodborne infections at both state and federal levels in order to identify foodborne outbreaks in a timely manner whenever they occur so as to implement timely control measures meant to keep the public safe. The outbreaks reported for the period in review were few due to the introduction of the zero tolerance policy of *L. monocytogenes* in RTE meats in 1989 by the USDA/FSIS and the subsequent requirement that facilities producing RTE products implement HACCP programs. These measures have been compounded by States enacting laws on the recommendation of the guidelines in the food code that have enhanced RTE meat consumer safety.

REFERENCES

1. Almeida, P. F., R. C. C. Almeida. 2000. A PCR protocol using *in*l gene as a target for detection of *Listeria monocytogenes*. *Food Control*. 11: 97-101

2. Andrews, W. H., and T. S. Hammack. 1998. *Salmonella. In* Bacteriological Analytical Manual Online. Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Gaithersburg, MD. Available at:. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalytical ManualBAM/UCM070149Retrieved 5/10/2011

3. Anthony, D. H., J. Karen. 2011. Detection and enumeration of *Listeria monocytogenes. In* Chapter 10 of the Bacteriological Analytical Manual Online. Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Gaithersburg, MD. Available at:http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalytic alManualBAM/ucm071400.htm Retrieved 3/2/2012

4. Anthony. D. H. 2011. A Meta-analytical estimation of the detection limits of methods for *Salmonella* in foods. *Food Research International*. 45: 1065-1071

5. Aznar, R., Alcaron. B. 2003. PCR detection of *Listeria monocytogenes,* a sample of multiple factors affecting sensitivity. *Journal of Applied Microbiology* 95: 958-966

6. Bohaychuk, V.M., G.E. Gensler, R.K. King, K.I. Manninen, O. Sorensen, J.T. Wu, M.E. Stiles and L.M. McMullen. 2006. Occurrence of Pathogens in raw and ready to eat meat and poultry products collected from the retail market place in Edmonton, Alberta, Canada. *J. Food Prot*. 69: 2176-2182

7. Brosch, R., M. Brett, B. Catimel, J.B. Luchansky, B. Ojeniyi, J. Rocourt. 1996. Genomic fingerprinting of 80 strains from the WHO multicentre international typing study of *Listeria monocytogenes* via pulsed-field gel electrphoresis (PFGE). *International Journal of Food Microbiology*, 32: 343–355

8. CDC. 2009. Salmonellosis. Available at: http://www.cdc.gov/nczved/divisions/dfbmd/diseases/salmonellosis/technical.html Retrieved 2/22/2012

9. CDC. 2010. FoodNet Reports. Availabe at: http://www.cdc.gov/foodnet/reports.htm. Retrieved 3/12/2012

10. CDC. 2010. *Salmonella*. Available at: http://www.cdc.gov/*Salmonella*/general/technical.html Retrieved 2/22/2012

11. CDC. 2011. Foodborne Illness Surveillance, Response, and Data systems. Available at: http://www.cdc.gov/foodborneburden/surveillance-systems.html Retrieved 3/12/2012

12. CDC. 2011. Listeriosis. Available at: http://www.cdc.gov/listeria/definition.html Accessed 2/22/2012

13. CDC. 2011. Nationally Notifiable Conditions. Available at: http://www.cdc.gov/osels/ph_surveillance/nndss/PHS/infdis.htm Retrieved 3/26/2012

14. CDC. 2012. Foodborne Outbreak Online Database. Available at: http://wwwn.cdc.gov/foodborneoutbreaks/ Retrieved 3/9/2012

15. CDC. 2012. Listeriosis Surveillance. Available at: http://www.cdc.gov/listeria/surveillance.html Retrieved 3/23/2012

16. Czachor. J. S. 1998. A brief Overview of Foodborne Illness. *Antimicrobials and infectious diseases newsletter*. 17: 73-75

17. Delaune, E., T. Sokol, R. Ratard, L. Allen, B. Kissler, S. Seys, K. Holt, P. Evans, T. Ward, B. Silk, K. Jackson, L. Graves, E. Trees, B. Silk. 2010. Outbreak of Invasive Listeriosis Associated with Consumption of Hog Head Cheese. Morbidity and Mortality Weekly Report. 60: 401-405

18. Dong, Y. L. 2010. Biology and Pathogenesis of *Salmonella*, p. 447-448. *In* Molecular detection of foodborne pathogens. CRS Press, Taylor and Francis Group, Boca Raton Florida

19. Dong, Y. L. 2010. Classification, Epidemiology, and Pathogenesis of *Listeria,* p. 207-211. *In* Molecular detection of foodborne pathogens. CRS Press, Taylor and Francis Group, Boca Raton Florida

20. Dong, Y. L. 2010. Conventional, Molecular, and Immunological Diagnosis of *Salmonella,* p. 447-455. *In* Molecular detection of foodborne pathogens. CRS Press, Taylor and Francis Group, Boca Raton Florida

21. Dong, Y. L. 2010. Nucleic acid detection methods, p. 3-7. In Molecular detection of foodborne pathogens. CRS Press, Taylor and Francis Group, Boca Raton Florida

22. Douglas, M.F, R. Zweig, J. Sturgeon, M. Tormey, M. Lecavalier, I. Lee, L. Lawani, L. Mascola. 2002. An Outbreak of Febrile Gastroenteritis Associated with Delicatessen Meat Contaminated with *Listeria monocytogenes*. *Clinical Infectious Diseases* 35: 943-949

23. Ellingson, J.L.E., J.L. Anderson, S. A. Carlson, V.K. Sharma. 2003. Twelve hour real-time PCR technique for the sensitive and specific detection of *Salmonella* in raw and ready to eat meat products. *Molecular and Cellular Probes* 18: 51-57

24. Endrikat. S., Gallagher. D, R. Pouillot, H. Quesenberry, D. Labarre, C.M.Schroeder, J. Kause. 2010. A comparative risk assessment for *Listeria monocytogenes* in prepackaged versus retail-sliced deli meat. *J. Food Prot*. 73: 612-9

25. Farber, J. M., and P. I. Peterkin. 2000. Listeria, p. 1178-1232. *In* B.M. Lund, A. C. Baird-Parker, and G. Gould. The microbiology of food. Chapman and Hall, London

26. FDA. 2009. Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins Handbook; *Salmonella*spp. Available at: http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/ucm069966.htm.   Retrieved 1/26/2012

27. FDA. 2012. FoodCode. Available at: http://www.fda.gov/Food/FoodSafety/RetailFoodProtection/FoodCode/default.htm Retrieved 3/26/2012

28. Fey, A., S. Eichler, S. Flavier, R. Christen, M. G. Hoffler, and C. A. Guzman. 2004. Establishment of a real time PCR-based approach for accurate quantification of bacterial RNA targets in water, using *Salmonella* as a model organism. *Appl Environ. Microbiol* 70 (6) 3618-23

29. FSIS. 2003. FSIS Final Rule Designed to Deduce *Listeria monocytogenes*in RTE meat & Poultry. Available at: http://www.fsis.usda.gov/oa/background/lmfinal.htm Retrieved 3/26/2011

30. Gombas, E. D., C. Yuhuan, C. S. Rocelle, and S. N. Virginia. 2003. Survey of *Listeria monocytogenes* in Ready to Eat Foods in the United States. *J Food Prot*. 66: 559-569.

31. Gottlieb S. L, E. C. Newbern, P.M. Griffin et al. 2006. Multistate outbreak of listeriosis linked to turkey deli meat and subsequent changes in US regulatory policy. *Clin Infect Dis*. 42:29-36

32. Gracias, K.S., J.L. McKillip. 2004. A review of conventional detection and enumeration methods for pathogenic bacteria in food. *Canadian Journal of Microbiology*. 50: 883-890

33. Graves, L. M., B. Swaminathan. 2001 PulseNet Standardized Protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed field gel electrophoresis. *International Journal of Food Microbiology*. 65: 55-62

34. Horwitz, W., G. W. Latimer. 2005. Isolation and Identification of *Salmonella* and *Listeria monocytogenes*, p. 117-230 *In* Official Methods of Analysis of AOAC International, Ch. 17 (18th Ed), AOAC International, Geithersburg, MD 20877-2417, USA

35. James, M. J. 2000. Physical, Chemical, Molecular, and Immunological Methods. Pulsed Field Gel Electrophoresis, p. 220. *In* Modern Food Microbiology (6[th]Ed), Aspen Publishers, Maryland, USA

36. Jones, R.C., V. Reddy, L. Kornstein, J. R. Fernandez. F. Stanvinsky, A. Agasan, S.L. Gerber. 2004. *Salmonella enterica* serotype Uganda infection in New York and Chicago. *Emerging Infectious Diseases*. 10: 1665-7

37. Julian, E., K. MacDonald, N. Marsden-Haug, L. Saathoff-Huber, R. Bonavolante, S. Otero, J. Nosari, C. Austin, D. V. Stein, A. Garvey, G. Kline, C. Lord, R. Groepper, B Kissler, M Parish, D. Elder, H. King, J. Pringle, J. Besser, S. Brown, K. Cooper, S. Sodha, I. Williams, C. Barton, L.G. Bettencourt. 2010. *Salmonella* Montevideo Infections Associated with Salami Products Made with Contaminated Imported Black and Red Pepper, United States, July 2009-April 2010. Morbidity and Mortality Weekly Report.59: 1647-1650. Also available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5950a3.htm Retrieved 3/10/2012

38. Khaitsa, M.L., B. Kegode, and D. Doetkott. 2007. Occurrence of antimicrobial resistant *Salmonella* species in raw and ready to eat turkey meat products from retail stores in the Midwestern United States, *Foodborne Pathogens and Disease* 4: 517-525

39. Levine, P., B. Rose, S. Green, G. Ransom, and W. Hill. 2001. Pathogen testing of ready to eat meat and poultry products collected at federally inspected establishments in the United States, 1990 to 1999. *J. Food Prot*. 64:1188-1193.

40. Lianou, A., J.N. Sofos. 2007. A Review of the Incidence and Transmission of*Listeria monocytogenes* in Retail and Food Service Establishments. *J Food Prot.* 70: 2172-2198

41. Marlony, B., J. Hoorfar, M. Hugas, A. Heuvelink, P. Fach, L. Ellerbrock, C. Bunge, C. Dorn. R. Helmonth. 2003. Inter-laboratory diagnostic accuracy of *Salmonella* specific PCR-based method. *Int Food Microbiol* 89(2-3): 241-9

42. McNabb, J. M, R.A Jajosky et al, 2005. Centers for Disease Control and Prevention Summmary of Notifiable Diseases. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5453a1.htm Retrieved 3/9/2012

43. Mead, P. S., L. Slutsker, V. Dietz, L F. Mccaig, J. S. Bresee, C. Shapiro, P. M.Griffin, R. V. Tauxe. 1999. Food Related Illness and Death in the United States. *Emerging Infectious Diseases*. 5: 607-625

44. Montville, T. J, and R. M. Karl. 2008. Application and Specific Proposals for Microbiological Criteria for Food and Food ingredients, p. 89. *In* Food Microbiology; an Introduction, (2nd Ed), ASM Press, Washington DC

45. Morbidity Mortality Weekly Report, CDC.2002. Public Health Dispatch: Outbreak of Listeriosis North Eastern United States. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5142a3.htm Retrieved 9/1/2011

46. Myint, M.S., Y.J. Johnson, N.L. Tablante, R.A. Heckert. 2006. The effect of pre-enrichment protocol on the sensitivity and specificity of PCR for the detection of naturally contaminated *Salmonella* in raw poultry compared to conventional culture. *Food Microbiol*. 23: 599-604

47. Newton, T., M.J. Jones. 2008. Iowa Surveillance of Notifiable and other Diseases Report. Available at: http://www.idph.state.ia.us/Cade/Default.aspx Retrieved 3/12/2008

48. O'Grady, J., S. S. Balbas, M. Maher, T. Smith and T. Barry. 2008. Rapid real time PCR detection of *Listeria monocytogenes* in enriched food Samples based on ssrA gene, a novel diagnostic target. *Food Microbiology*.1: 75-84

49. Olsen, J.E., S Aabo, W. Hill, S Notermans, K. Wernars, P.E. Granum, T. Popovic, H. N Rasmussen, O. Olsvik. 1995. Probes and PCR for Detection of foodborne bacterial pathogens. *Int.J. Food Microbiol.* 28: 1-78

50. Patel. J.R., Bhagwat. A.A. 2008. Rapid real time PCR assay for detecting *Salmonella* in raw and ready to eat meats. *Acta Vet Hunga*. 56: 451-8

51. Pradhan A.K., Ivanek. R, Y.T. Grohn, R. Bukowski, M. Wiedmann. 2011. Comparison of public health impact of *Listeria monocytogenes* product to product and environment to product contamination of deli meats at retail. *J. Food Prot*. 74: 1860-8

52. Ribot, E. M., Fair. M. A, Gantum. R, Cameron. D. N, Hunter. S. B, Swaminathan. B, Barrett. T. J. 2006. Standardization of Pulsed-Field Gel Electrophoresis Protocols for the Subtyping of *Escherichia coli* 0157:H7, *Salmonella*, and *Shigella* for PulseNet. *Food-borne Pathogens and Disease.* 3:59-67

53. Ryser, E. T., and E. H. Marth. 1999. The genus *Listeria* and *Listeria monocytogenes*, and Listeriosis p. 8-57. *In Listeria,* Listeriosis, and Food Safety (3rd Ed), CRC Press, Boca Raton, Florida, US.

54. Scallan, E., R.M. Hoekstra, F.J. Angulo, R.V.Tauxe,M.A. Widdowson, S.L.Roy. 2011.Foodborne illness acquired in the United States, major pathogens. *Emerg Infect Dis*. 17:7-15

55. Scallan. E., P. M. Griffin, F.J. Angulo, R. V. Tauxe, R. M Hoekstra, M.C. Widdowson, L.R. Sharon and L.J. Jeffery. 2011. Foodborne illness acquired in the United States, unspecified agents. *Emerg Infect Dis* 2011.17:16-22

56. Sonja, J. O., M. Patrick, S.B. Hunter et al. 2005.Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. *Clinical Infectious Diseases.* 40: 962-967

57. Uesugi, A.R., C.I. Moraru. 2009. Reduction of Listeria on ready to eat sausages after exposure to a combination of pulsed light and nisin. *J. Food Prot*. 72: 347-53

58. United States Department of Agriculture (USDA). 1989. Revised policy for controlling *Listeria monocytogenes*. Federal Register. 54:22345-6.

59. USDA/Food Safety and Inspection Service (FSIS). 2002. History of HACCP based Inspection Models Project. Available at: http://www.fsis.usda.gov/Science/HIMP_History/index.asp Retrieved 3/26/2012

60. USDA/FSIS. 2007. Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry, Egg and Environmental Samples; Laboratory guide book. http://origin-www.fsis.usda.gov/PDF/MLG_8_07.pdf. Retrieved 5/26/2011

61. USDA/FSIS. 2008. Isolation and Identification of *Salmonella* from Meat, Poultry, and Egg products. Available at: http://www.fsis.usda.gov/PDF/MLG_4_05.pdf Retrieved 2/23/2012

62. USDA/FSIS. 2010. The FSIS microbiological Testing Program for Ready to Eat Meat and Poultry Products. Available at: http://www.fsis.usda.gov/Science/Micro_Testing_RTE/index.asp Retrieved 3/26/2012

63. Varma, J.K., M.C.Samuel. R. Marcus. R.M.Hoekstra. C. Medus, S. Segler, B.J. Anderson, T.F. Jones, B. Shiferaw, N. Haubert, M. Megginson, P.V. McCarthy, L.

Graves, T.V. Guilder, and F.J. Angulo, 2006. *Listeria monocytogenes* Infection from

foods prepared in commercial establishments: A case control study of potential sources of

sporadic illness in the US. *Clinical infectious Diseases* 44: 521-528

64. Wenger, J.D., B. Swaminathan, P.S. Hayes, S.S. Green, M. Prat, R.W. Pinner, A.

Schuchat, C.V. Broome. 1990. *Listeria monocytogenes* contamination of turkey franks:

Evaluation of a production facility. *J Food Prot*. 53:1015-9

65. Wolffs, P.F., K. Glencross, R. Thibaudeau, M.W. Griffiths. 2006. Direct quantification

and detection of *Salmonellae* in biological samples without enrichment using two step

filtration and real time PCR. *Appl Environ Microbiol.* 72: 3896-900

66. Yang, H., A. Mokhtari, L.A. Jaykus, R.A. Morales, S.C Cates, P. Cowen. 2006.

Consumer phase risk assessment for *Listeria monocytogenes* in deli meats. *Risk Anal*. 26:

89-103