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Prevalence of *Salmonella* species and *Escherichia Coli* O157:H7 in organically managed cattle and food safety status of selected meat products

by

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Kathleen Delate

The student author and the program of study committee are solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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NOMENCLATURE

E.coli O157:H7	*Escherichia coli* O157:H7
CFU	Colony Forming Units
Salmonella spp.,	*Salmonella* species
g	Gram
CFR	Code of Federal Regulations
RTE	Ready-to-eat
FSIS	Food Safety and Inspection Service
USDA	United States Department of Agriculture
STEC	Shigatoxigenic *Escherichia coli*
LAB	Lactic Acid Bacteria
MIC	Minimum inhibitory concentration

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ABSTRACT

Beef is an important source of food for consumers, thus requiring the safety of beef products important when considering the potential for a foodborne related illness. The food safety status of ground beef and fermented sausage products was examined in the literature along with interventions applied to reduce food safety risk. Ground beef was found to be a more important contributor to foodborne illnesses traced back to beef, and, undercooked ground beef was frequently associated with *E.coli* O157:H7 outbreaks. *Salmonella* spp., and *E.coli* O157:H7 are frequently associated with ground beef. Fermented sausage products were found to be a low risk food as the manufacturing process includes several hurdles for microbial growth. In fact, most outbreaks related to fermented sausages occurred due to post-processing contamination which signifies the importance of proper implementation of food safety practices in production and food service environments.

With the increasing growth in the sales and market share of organic goods it is imperative to look at the safety aspects of such production methods. Many organic production systems rely on manure in order to build soil organic matter and improve fertility. Manure has, however, been shown to be an ideal vehicle for transfer of pathogens like *E.coli* O157:H7 and *Salmonella* spp., and studies have shown that these pathogens can be successfully transferred onto vegetation. This brings into question the safety of such systems. The growth rate of organic sales is increasing yearly along with the potential of outbreaks of

possible foodborne illnesses, making it imperative to study the food safety aspects of these systems of production.

In a multi-state project that ran from 2015-2017 cattle were raised using an integrated in Iowa, Minnesota and Pennsylvania. Fecal, feed, hide and meat samples were obtained and analyzed for the presence of *E.coli* O157:H7 and *Salmonella* spp. Analysis was carried out using the miniVidas system developed by bioMérieux and confirmatory tests were performed according to USDA and FDA BAM procedures.

Prevalence rates for *E.coli* O157:H7 and *Salmonella* spp., were comparable or numerically lower when compared to other similar organic studies. Occurrence of these pathogens was similar to conventional systems as well. Overall, *E.coli* O157:H7 was present in 9.43% of feed samples and 7.26% of fecal samples. There was no *E.coli* O157:H7 detected on hide or meat samples. *Salmonella* spp., was found in 1.89% of feed samples, 3.26% of fecal samples and 18.6% of hide samples. No meat sample was positive for *Salmonella* spp. The occurrence of either pathogen was very low and was found to be similar to levels in the region or in previous studies.

CHAPTER 1: INTRODUCTION

The sale of organic goods in the United States has been increasing every year. The Food Marketing Institute and North American Meat Institute showed in 2015 that meat and poultry sales have gone up by 29.3% (Sustainable Food News, 2016). The penetration of organic goods in households in 2015 was 6.5% up by 1.3% in 2014 (Sustainable Food News, 2016). There is a large potential for growth in organic market sales as shown in 2015 when sales reached $43 billion (Watrous, 2016).

Consumers have several reason for choosing organic, such as perceived health benefits, ethical values, quality, perceived safety of food, education level, ethnicity and even age (Dimitri and Dettmann, 2012; Michaelidou and Hassan, 2008). Organic crops and livestock are raised without usage of pesticides and antibiotics, an aspect which is part of the attraction consumers have towards organic food. Organic producers are allowed to use naturally occurring chemicals and the list of allowed chemicals can be found in "The National List of Allowed and Prohibited Substances." ACNielsen conducted a survey amongst U.S. consumers and it was found that they associate organic food with a better nutrient profile and richer taste (ACNielsen, 2005) although, in a study conducted on beef taste no difference was found in a blind taste test (F. Napolitano et al., 2010). Consumers are willing to pay more for organically produced food due to all these perceived superior qualities (Didier and Lucie, 2008; F. Napolitano et al., 2010; Fabio Napolitano et al., 2010). A review of over 150 studies based in Europe however found the nutritional and sensory claims to be highly variable as the methods of evaluation of physiochemical properties and sensory tests showed no

difference or were contradictory (Woese et al., 1997). Nonetheless the label "organic" has an impact in at least 50% of consumers with a good proportion of them striving to buy organically labelled goods even if it meant paying a premium (Didier and Lucie, 2008).

In a study by Harvey et al., consumers perceived organic food as safer even though foodborne outbreaks have been associated with organic products (Harvey et al., 2016). Specifically, Harvey et al.,2016, found that as sales increased so did the likelihood of an outbreak. This is something to take into serious consideration as organic practices can involve soil amendments with manure which is known to be an ideal vehicle for delivering major foodborne pathogens like *Escherichia coli* O157:H7 and *Salmonella* species (Jiang et al., 2015; Kudva et al., 1998). *E.coli* O157:H7 originating from manure has also been found in lettuce and parsley (Islam et al., 2004). Transmission of the aforementioned pathogens from manure to pasture and grazing animals has also been documented (Holley et al., 2007). Overall these studies indicate that organic methods may not produce food that is safe and free from pathogens if not managed effectively with some studies showing no difference in the safety status of food produced by both organic and conventional methods (Fegan et al., 2004; Kijlstra et al., 2009; Reinstein et al., 2009).

An important change detected in consumer behavior was that ethical identity is now becoming an important factor when it comes to decision making (Brennan et al., 2003; Didier and Lucie, 2008; Hermansen, 2003). Organic livestock methods are based on animal welfare requiring year-round access to the outdoors and grazing (Coffey and Baier, 2012) and methods that confer "happier, healthier" and "more humane" treatment of animals which appeal to consumers. The forage based diets animals have on pasture may expose them to

more sources of contamination due to increased exposure to sources like wildlife and insects (flies) (Sinton et al., 2007; Wilkes et al., 2013) but, the diet may confer some protection against pathogenic bacteria (Jacobson et al., 2002), although, studies are contradictory on this aspect (Baale et al., 2004). The study by Jacobson et al.,2002, examined *E. coli* in fecal matter and rumen content of in cattle on a forage diet and found them to be lower than grain-fed cattle whereas, Baale et al.,2004, found that cattle on forage diets shed *E.coli* O157:H7 in their feces for extended periods.

Sustainability practices undertaken by industry have now also become a part of the ethical aspect of choosing organic products (Michaelidou and Hassan, 2008). Organic production systems have found to have fewer impacts on the environment (Tuomisto et al., 2012) and tend to be seen by consumers as environmentally friendly. Organic livestock production may be more expensive per unit input (Sundrum, 2001) as it requires greater land mass and has animal densities lower than conventional systems, making optimization mandatory for organic livestock growers.

Consumer expectations for organic foods are higher than for conventional foods with consumers willing to pay a premium (Napolitano et al., 2010; Napolitano et al., 2010). The reasons for this include ethical, health, perceived food safety and quality being major driving forces for organic adoption. From a sustainable aspect, organic methods do have a positive environmental impact which may resonate with a greater proportion of consumers as concepts like global warming become more widely accepted and acknowledged. Agencies do have a responsibility to educate consumers about food safety risks as a common misconception among consumers is organic food is safer than conventional with respect to

foodborne pathogens. Further research into the effect of diet must be conducted as current research involves a lot of variability and contradictions.

1.1 Pathogens associated with selected beef products and interventions applied to ensure food safety

Beef is obtained from an animal source and, may contain pathogens which leads to outbreaks of foodborne illnesses in humans (Crump et al., 2002; Hussein and Bollinger, 2005). From 1998-2008 beef was estimated to be the source of 6.6% of foodborne illnesses in which the etiological agent was determined (approximately 7998) and responsible for 12% of outbreaks (Gould et al., 2013) and illnesses resulting from consumption of meat and poultry was at 43% (Painter et al., 2013). Painter et al., 2013, also found that meat and poultry derived products were frequently implicated in bacterial related outbreaks. The pathogens generally associated with raw or undercooked beef include but are not limited to: pathogenic *E.coli*, *Salmonella* spp., and *Campylobacter jejuni* (Crowe et al., 2015; Fox et al., 2008; Schlundt, 2002).

Of all beef products ground beef is often linked to foodborne outbreaks and pathogens such as *E.coli* O157:H7, *Salmonella* spp., have been detected in ground beef across the U.S. (Hussein and Bollinger, 2005; LeJeune and Christie, 2004; Samadpour et al., 2006). The low infectious doses of some pathogens like *E.coli* O157:H7 and its ability to replicate under temperatures as low as 7°C (Tamplin, 2002; Tamplin et al., 2005) make even low levels of pathogens in retail products extremely dangerous.

Fermented sausages have been found to be relatively safer and of lower risk associated with foodborne illnesses as the processing involves several hurdles for microbes

such as nitrates, sodium chloride, heat, lactic acid and lactic acid bacteria, phenols and low water activity (Adams and Mitchell, 2002). The pathogens commonly associated with fermented sausage include some strains of verotoxigenic *E.coli, Salmonella* spp., *Staphylococcus aureus* and *Listeria monocytogenes* (Adams and Mitchell, 2002; Adams and Nicolaides, 1997; Farber et al., 1993). Since many sausages are considered Ready-to-eat (RTE) foods the FSIS mandates that all fermented sausage meet a 5log reduction and also a minimum number of degree hours in order to produce pathogen free food.

In order to ensure safety of products many interventions are applied such as use of essential plant oils, organic acids, lactic acid bacteria, packaging and cooking (Adams and Mitchell, 2002; Bajpai et al., 2012; E. Mani-López et al., 2012; Kudra et al., 2013b; Sultana et al., 2014; Sung et al., 2013). The use of natural extracts from plant oils like garlic were used to not only control microbiological activity but also enhance flavor (Sung et al., 2014). The major problem with plant essential oils and organic acids is that they severely affect flavor and so applications may be limited (Morey et al., 2014; Smulders and Greer, 1998). Packaging systems like Modified Atmosphere Packaging (Gunes et al., 2011; Kudra et al., 2013) are currently being employed to control microbial growth. Newer packaging technologies include embedding anti-microbial components in the packaging system itself but these are still under development and not used extensively (Sung et al., 2013).

Overall outbreaks of ground beef are caused due to undercooked products though certain non-O157 STEC have been shown to be capable of surviving cooking temperatures (Pokharel et al., 2016). The low risk associated with sausages is reflected by the fact that since FSIS implemented the 5log reduction law in 1994 there have not been any major outbreaks.

The outbreaks that have occurred have been related to post-processing contamination or improper hygiene (Gormley et al., 2010). Packaging is also starting to play an important aspect in food safety and will probably assume a greater role in the future.

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CHAPTER 2: LITERATURE REVIEW: CONSUMER PERCEPTION OF ORGANIC FOOD AND PREVALENCE OF *SALMONELLA* SPP. AND *E. COLI* O157:H7 IN ORGANICALLY RAISED CATTLE

2.1 Introduction

Organic sales are at an all-time high. There was a 72% increase in organic sales from 2008 to 2014 (Young, 2015). The total value of organic sales from farms (exempt and non-exempt) in 2014 was around $5.5 billion (Young, 2015). Additionally, the National Agriculture Survey Service (NASS), reported that in 2014 around 688 farm were getting ready to transition to organic systems and another 39% of current organic farms are expanding (USDA-NASS, 2012). All organic farming systems have to follow strict rules and regulations as laid out by the National Organic Program (Coffey and Baier, 2012).These include no use of synthetic chemicals, antibiotics and other practices. Farmers also receive a premium on cattle head that are organic. Whilst organically managed cattle cannot be given antibiotics and, those that fall ill must receive treatment and be shifted to conventional systems once treated with antibiotics. Farmers can opt to treat cattle using "alternative methods" (Coffey and Baier, 2012) using approved chemicals but these are not always effective.

Demand for organic goods has increased year after year; there was a $3.9 billion increase in 2014, and a $4.2 billion increase in 2015 (Watrous, 2016). The Food Marketing Institute and North American Meat Institute saw a growth of 29.3% in sales of organic meat and poultry in 2015 (Sustainable Food News, 2016). There is still room for growth in the organic sector according to the Organic Trade Association as penetration of organic goods was found to be 6.5% in 2015 compared to 5.2% in 2014 (Sustainable Food News, 2016).

Ethical self-identity, perceived food safety and, attitude and intention, with ethical self-identity superseding all factors including health (Michaelidou and Hassan, 2008).

Consumers associate organic with higher food quality and greater safety. A comprehensive review of over 150 food types was conducted in Europe in 1997 showed that consumers rated organic food higher due to non-usage of chemicals, banned use of genetically modified organisms and, better nutrient profile. Upon analysis, it was shown that organic food and conventional food have the same food safety status (Harvey et al., 2016). Pesticide residue in conventional crops was much below statutory limits. The findings on nutritional profiles were however contradictory (Woese et al., 1997).

2.3 Organic Livestock Production

Organic systems are very different from conventional systems in that they approach the farm environment as a whole rather than be yield-oriented. The Soil Association which is credited to be the largest promoter of soil health is an international organization and summarizes organic farming values as follows: coexist with nature, improve and build soil, ethical treatment of animals, augment and better the farm environment, have a more positive social and ecological impact.

These values which focus on ethics are what resonate with adopters of organic foods and have now become a driving factor in acceptance of organic systems and products (Michaelidou and Hassan, 2008).

The National Organic Program, which runs under the USDA, has several regulations with respect to organic raising of livestock. The legal intricacies are defined in Title 7, Part 205

animals. When animals get sick they can be subject to alternative treatments or be treated with conventional medicines. Once an animal is treated with conventional medicine it is no longer classified as organic. Feed and pasture lands must also be organically certified.

iii.   Crop and forage land be certified organic

Crops and forage land used to provide feed for the livestock must be certified organic, that is, they must be organically managed. If chemicals need to be used they must be approved by the farms Certification Organization before application.

iv.   Unrestricted access to the outside environment year-round

USDA regulations prohibit the continuous confinement of livestock in organic systems. Livestock should be given year-round access to the outdoors. Confinement of livestock is allowed under certain situation like extreme weather. During the grazing season livestock is expected to be out on pasture daily and should meet the grazing requirement of a 120 days per calendar year. The pasture and forage land should not have its carrying capacity exceeded under any circumstance.

v.   Maintenance of an Organic System Plan (OSP)

The Organic System Plan is the most important document for any organic operation. It is required by law (§ 205.201 a) to list a description of farm practices, inputs (chemicals, feed, etc), monitoring and verification, record keeping methods and plan, and the setup and management of buffer zones to prevent co-mingling of conventional and organic products. The certifying agency can also ask for other details which they deem necessary to ensure compliance with standards.

vi.   Usage of chemicals (OMRI approved chemicals can be used in general) and antibiotics is not allowed.

The National List of Allowed and Prohibited Substances defines and limits what inputs can and cannot be used in organic operations. OMRI (Organic Materials Review Institute) evaluates products for use in organic systems. OMRI listed products can in general be used by organic farmers but since OMRI is not a regulatory body producers should always check with their certifiers before using any chemical. Antibiotics cannot be used in organic livestock production.

2.4 *E.coli* O157:H7 and *Salmonella* spp., in Organic Livestock Systems

Despite consumer beliefs that organic products are safe several outbreaks linked to organic foods have been reported (Harvey et al., 2016). Harvey et al, studied 18 outbreaks irrevocably linked to organic foods from 1992 to 2014 *Salmonella* species and *E.coli* O157:H7 were responsible for 44% and 33% of the outbreaks associated with the 18 linked to organic foods. In comparison there were a total of 7,246 confirmed viral or bacterial foodborne related outbreaks from 1998-2008; with an increase post 2005 (Gould et al., 2013).The high proportion of outbreaks caused by *E. coli* O157:H7 and *Salmonella* spp., in meat products is why these two pathogens are tested for. Over half the recorded outbreaks occurred from 2010 to 2014 and this upward trend matches the increased growth of the organic sector and implies that organic food may not be as microbiologically safe as assumed.

Organic farming systems rely on the application of manure and crop rotation as well. Usage of pasture in the rotation or improperly composted manure could lead to deposition

of *Salmonella* and *E.coli* O157:H7 in the soil which can then be transferred to vegetation grown (Franz et al., 2005; Fremaux et al., 2008; Jacobsen and Bech, 2012). Several studies have shown how pathogens can be transferred to lettuce and parsley leaves via manure (Islam et al., 2004).

Although organic systems do not use antibiotics, there have been studies that have isolated antibiotic resistant strains in cattle raised in organic systems (Miranda et al., 2009; Reinstein et al., 2009). Reinstein et al., 2009, not only found antibiotic resistant strains in both systems but found no significant difference in their occurrence in organic and conventional systems. The presence of antibiotic resistant strains is of grave concern as antibiotics are used to treat bacterial infections. Antibiotic strains render current antibiotic treatments useless and this might lead to an elevation in deaths or illnesses caused due to bacterial pathogens.

The increase in organic sales along with the upward number of outbreaks traced back to organic products (Harvey et al., 2016) and, the presence of antibiotic resistant strains in cattle imply that greater efforts must be taken in the farm and processing environments to ensure a safe food supply.

2.4.1 *E. coli* O157:H7 in cattle

2.4.1.1 Introduction

Beef has been particularly notorious for being the source of many outbreaks of diarrhea associated with *E. coli* O157:H7 (Crump et al., 2002; Gyles, 2007; Hussein, 2007) and in fact due to the significant increase of cases during the grilling season (Rangel et al., 2005) *E. coli* O157:H7 infection is colloquially known as "Hamburger Disease." In fact beef is

associated with 47% of confirmed outbreaks; with 41% being attributed to ground beef and 6% to other beef cuts (Rangel et al., 2005). Beef therefore is the single most common food implicated in *E.coli* O157:H7 outbreaks and is therefore a major cause of concern in the beef industry be it conventional or organic (Harvey et al., 2016).

E.coli O157:H7 has been frequently isolated from the farm environment and is ubiquitous (Davis et al., 2005; Dodd et al., 2003; Lynn et al., 1998; Matthews et al., 2006; Sheng et al., 2015).; Feed has been implicated as a source (Berry and Wells, 2010; Hancock et al., 1998; Lynn et al., 1998) that, not only acts as a vehicle but could possibly allow for replication (Lynn et al., 1998). Authors also tend to disagree as to whether indication of generic coliform counts can be taken to represent possible presence of O157 as it is thought that other coliforms may outcompete *E.coli* O157:H7 in terms of growth.(Chapman et al., 2001; Dodd et al., 2003). Most literature also frequently states that the detection and prevalence of *E.coli* O157:H7 is under-represented in most studies due to lack of sufficient herd sizes, sampling points or techniques employed (Byrne et al., 2003; Chapman et al., 2001; Chapman and Ashton, 2003; Gansheroff and O'Brien, 2000).

A stated earlier several sources have been implicated with feed, and fecal matter being the most common followed by water, flies and wildlife (Berry and Wells, 2010; Hancock et al., 1998; LeJeune et al., 2001; LeJeune and Wetzel, 2007). Since organic farming systems mandates that livestock get a minimum of a hundred and twenty days on pasture (Coffey and Baier, 2012) they may be exposed to one or more of these sources of contamination. Most studies look at feedlots and conventional systems or cattle at abattoirs which is why this is a

unique study, at the moment. In order to better understand *E.coli* O157:H7 on the farm this section will be broken down into further subsections.

2.4.1.1 Sources of *E.coli* O157:H7

Sources of *E. coli* O157:H7 include: feed, pasture, water, manure, wildlife, the farm environment (pens, bedding, other cattle), hide. The sources will be broken in sections to be discussed further.

2.4.1.2.1   Feed

As stated earlier feed is frequently found to be a source of contamination in all types of cattle operations (Berry and Wells, 2010; Cobbaut et al., 2008; Dargatz et al., 2005; Davis et al., 2003; Krytenburg et al., 1998). This suggests that feed may be a starting point to control *E.coli* O157:H7 on farms. *E.coli* O157:H7 has been found to frequently replicate in feed as seen in warmer months with a 1.5 log increase in 48 hours at 21°C (Lynn et al., 1998). In organic farms bales, may be rolled and then left exposed to the elements for more than 48 hours; this presents an opportunity for the replication of *E.coli* O157:H7. Feed may be left the environment for any number of days and provided with the right input, that is, moisture and adequate heat, may replicate and build to much higher concentrations, which could effectively lead to inoculation of cattle. Adequate interventions such as length of time feed is allowed to stay idle, proper sanitation of feed bunks and possibly heat treatment of feed may be implemented to reduce the risk of feed being a potential vehicle for *E.coli* O157:H7.

2.4.1.2.2 Pasture

The National Organic Program mandates that organically raised cattle must spend a minimum of 120 calendar days on pasture (Coffey and Baier, 2012). Pastures are used for grazing and generally have greater biodiversity than cropland. The exposure of animals to the environment can also introduce several sources of contamination like wildlife, river-water, and fecal matter from other cows. Studies have shown that *E.coli* O157:H7 has the ability to survive on pasture in manure (Callaway et al., 2003; Edrington et al., 2010; Indira T Kudva et al., 1998; LeJeune and Wetzel, 2007; Russell et al., 2000; Sinton et al., 2007), and can be a significant source of contamination (Russell et al., 2000) , although, it seems that the type of manure applied (Holley et al., 2007) and seasonal variation does have an effect (Sinton et al., 2007) on the importance of this source. There seems to be a difference of opinion with respect to pastures contributing to *E.coli* O157:H7 transmission with some supporting the claim that pasture grazing reduces transmission (Baale et al., 2004; Callaway et al., 2003; Jacobson et al., 2002) but with others arguing that survival of the pathogen in pastures makes it an important factor in transmission (Muirhead, 2009; Rumpold and Schlüter, 2013; Sinton et al., 2007).

2.4.1.2.3 Water

It has been shown that water troughs can also act as sources of contamination as bacteria can survive in them due to presence of high organic load; saliva, chewed feed, dirt (Hancock et al., 1998; LeJeune et al., 2001; Sargeant et al., 2004; Shere et al., 2002). Studies sometimes use coliform counts as an indicator of contamination but this may not always be

accurate (Sanderson et al., 2005) as it is now seen that other coliforms may compete with *E.coli* O157:H7 for resources. There is a general trend where concentration increases in the warmer months (LeJeune et al., 2001) as seen with feed but LeJeune et al. used total coliform counts as an indicator for *E.coli*, which may not present an accurate representation. Other studies have found water to be minimally associated with transmission (Cobbaut et al., 2008), though this may be modified by presence of increased fecal contamination or temperatures above 15°C (Avery et al., 2008; LeJeune et al., 2001; McGee et al., 2002). Levels of pathogens in water are reported to be less than 1log cfu/ml (Avery et al., 2008, Lejune et al., 2001) but, repeated exposure via this route could lead to significant buildup and even colonization. Although the risk has to be firmly established by research. *E.coli* O157:H7 can also be carried by runoff water into streams which can then disseminate the organism to other pastures or areas downstream (Avery et al., 2008; Wilkes et al., 2013). The study by Wilkes et al., 2013 showed that *E.coli* O157:H7 survival is better in puddles and lakes (12 days) versus troughs and streams (7 days). Constant contamination of surface waters may therefore enable them to serve as potential reservoirs, though much more research is needed in this area.

2.4.1.2.4   Manure

E.coli O157:H7 is a serotype of Escherichia group which are enterobacteria that normally occupy the lower gut of mammals and are commonly excreted in fecal matter. Most Escherichia are symbionts and are present in the guts of bovine, higher mammals and even humans, which is why generic *E.coli* are used as indicator organisms for fecal contamination (LeJeune et al., 2001). *E.coli* O157:H7 however, is pathogenic and causes diarrhea along with

hemolytic uremia, particularly in immuno-compromised patients. Survival of pathogens in manure piles is much higher (Holley et al., 2007; I T Kudva et al., 1998) especially if not exposed to dry conditions (such as those found in the top layer of manure) or direct sunlight (UV exposure), both of which have a direct impact and lead to reduced survival rates (I T Kudva et al., 1998). Fecal shedding of *E.coli* O157:H7 in cattle in highly variable (Sanderson et al., 1999; Shere et al., 2002) with age, level of inoculation/colonization, stress, breed and even temperature playing important aspects. Many studies try to quantify shedding, which would be useful in its own right especially when it comes to developing preharvest controls, some studies look at the survival and horizontal transmission (LeJeune and Wetzel, 2007; Maciorowski et al., 2007) which can be easily measure and quantified. These studies concluded that manure is in fact an important factor that allows for organisms to persist in the farming environments and they also advocate for frequent cleaning of pens, change of bedding and even cleaning between herds. For organic systems, it was found that actively managed land had lower risks of transmission.

2.4.1.2.5   Wildlife

The term wildlife encompasses a wide variety of animals that can be present; deer, avian, rodents and even flies. Wildlife has been frequently associated with not only acting as a reservoir but also enabling migrations of strains over land (Hancock et al., 1998; LeJeune and Wetzel, 2007; Rice et al., 1995). Strains isolated from wildlife have been matched to strains found on farms in cattle (Rice et al., 1995) and avian wildlife, such as starlings which have a low prevalence of Shiga toxin producing *E.coli* (STEC) in their fecal matter, have in fact

been used to successfully isolate strains of *E.coli* O157:H7 that have been matched to cattle on farms via pulse-field electrophoresis (Wallace et al., 1997). Birds perch on cattle and come into contact with hides, which can contain fecal residue and therefore act as a vector, transmitting the pathogen across a wide geographical location. Rats have also been found to have identical strains isolated form cattle and they are generally a problem in feed barns/mills where they may get into barns and defecate on stored feeds (Nielsen et al., 2004). The contribution of insects is generally not taken into account though flies have been shown to act as vectors of *E.coli* O157:H7 (Rahn et al., 1997). Although cattle are considered as the primary reservoirs it is recommended to keep wildlife away from pastures in order to minimize risks or transmission across areas of land (LeJeune and Wetzel, 2007).

2.4.1.2.6 Farm environment (Pens, bedding, contact with other cattle)

The farm environment is also an important factor when it comes to maintaining a population of viable bacteria. Bacteria has been shown to survive on cage and pen surfaces, bedding and flooring and even crevices of water troughs (Davis et al., 2005; LeJeune et al., 2001; LeJeune and Wetzel, 2007; Sargeant et al., 2004). Replication in water troughs is dependent on temperature. Mixtures of urine and bedding have been found to enhance the growth of *E.coli* O157:H7 (Davis et al., 2005; LeJeune and Wetzel, 2007; Lynn et al., 1998) further underscoring the importance of clean pens and beds. Thorough cleaning is recommended between herds along with change of bedding and frequent removal of fecal matter to ensure that populations do not buildup and colonize the space. The type of bedding is also important as demonstrated by Davis et al., 2005 where cedar chip bedding moistened

with bovine urine was found to more frequently contain higher levels of *E.coli* O157:H7. Manure piles also allow for prolonged survival of the bacterium outside the host (Holley et al., 2007; I T Kudva et al., 1998) and should therefore be removed as frequently as possible.

2.4.1.2.7   Hide

E.coli O157:H7 has also been isolated on hides of cattle. This is mainly due to fecal contamination or dirt on the hide (Brichta-Harhay et al., 2008). It is interesting to note however that the detection of *E.coli* O157:H7 on hides is much lower than for fecal samples and as such it may not be a relevant indicator of contamination (Cobbaut et al., 2008). A study by Brichta-Harhay et al., 2008, however, found *E.coli* O157:H7 on 46.9% of hides sampled and no variation by season (Brichta-Harhay et al., 2008) which they explained by stated that the slaughter facilities they sampled from received cattle from a wide range of geographical locations and this might have eroded any difference due to seasonality. Arthur et al., 2011, determined that survival on hide was very short-lived and could detect presence only for up to nine days (Arthur et al., 2011). They also recommended that any intervention should be applied just prior to harvest as these will be most effective. The "visible dirtiness" of hide was also found to not be a useful indicator of contamination (Jacobson et al., 2002). The study by Jacobson et al., 2002, however examined generic *E.coli* instead of *E.coli* O157:H7 which might not be completely applicable as generic *E.coli* may outcompete *E.coli* O157:H7 or vice versa (Chapman et al., 2001; Dodd et al., 2003), though until a concrete establishment between the two populations has been established this can serve as a guide. Nonetheless hide contamination has been linked to carcass contamination by fingerprinting methods

(Aslam et al., 2003; Barkocy-Gallagher et al., 2001). The high variability of cattle within the same plant; 39-56% positive (Brichta-Harhay et al., 2008), implies that hide might be a significant source of contamination in some cases and in order to ensure that the food supply is kept safe it must be assumed that cattle have a prevalence of *E.coli* O157:H7 in the upper ranges thereby increasing the importance of pre-harvest and post-harvest sanitation steps (Arthur et al., 2011; Brichta-Harhay et al., 2008).

2.4.2   Prevalence of *E.coli* O157:H7 in cattle

The prevalence of *E.coli* O157:H7 is varied depending on geographical location, feed type and even breed. Rates of prevalence in feces are reported to be in the range of 9.3% (Reinstein et al., 2009) up to 47% in cows raised on a farm/ranch system (Van Donkersgoed et al., 1999).Global rates as reported were also found to vary from0.2 to 27.8%(H. H. S. Hussein and Bollinger, 2005). The high variability is thought to occur due to many factors such as: Season of sampling (Chapman et al., 2001; Hancock et al., 1994; Varela-hernández et al., 2007), Age of animal (Cobbold and Desmarchelier, 2000), Method of sampling (fecal pat, rectal swab, fecal grab, swabbing style, sample size and area) (Cobbaut et al., 2008; Lahti et al., 2003), Methods for detection and isolation(Chapman and Ashton, 2003), Geographical location and breed (H. H. S. Hussein and Bollinger, 2005; Hussein, 2007), State of animal (fed, fasted, stressed, diseased, shedders) (S. J. Buchko et al., 2000; Gregory et al., 2000), Diet (Baale et al., 2004), Presence of super shedders (LeJeune and Wetzel, 2007; Omisakin et al.,

2003), and, Presence of wildlife and other animals (LeJeune and Wetzel, 2007; Sasaki et al., 2011)

Every study follows different combination of the factors and, so it often very hard to compare between studies. Studies have shown that there is no difference between organically raised and conventionally raised cattle with respect to occurrence of *E.coli* O157:H7 (Miranda et al., 2009; Sofos, 2008). No significant differences between the occurrence of *E.coli* O157:H7 was also found between feedlot and pasture raised cattle (Fegan et al., 2004).The study by Byrne et al.,2003, in which dairy cattle from Iowa, Illinois and Wisconsin were sampled at a processing plant showed a rate of 4.9% in downer cattle versus 1.5% in healthy cattle (Byrne et al., 2003) thereby indicating that health status of cattle affects the shedding of *E.coli* O157:H7. The cattle were not stated to be organically certified but as stated earlier there have so far been no differences in occurrence of the pathogen so the rates between the two systems are comparable. Cows fasted overnight were shown to have higher *E.coli* counts (Gregory et al., 2000; Jacobson et al., 2002) due to the more neutral pH of the rumen. It has been shown that a fasting period of around 16 hours reduces the amount of *E.coli* O157:H7 shed (Harmon et al., 1999) but other studies have indicated contradictory results. Buchko et al., 2000, showed that if animals are fasted 48 hours then re-fed and fasted for another 48 hours there was in 42.8% increase in the shedding *E.coli* O157:H7 (S J Buchko et al., 2000). This is particularly important as animals have to be transported to slaughterhouses. It also shows the effect stress has on shedding of pathogens and could therefore be used as a potential control measure. Further studies should be

conducted to determine effect of diet and fasting on duration of shedding, in order to construct windows of opportunity to harvest safely.

Carcass levels depend to a large extent on: Method of Slaughter/ Slaughter house management practices (Arthur et al., 2002) , Geographical Location (H. S. Hussein and Bollinger, 2005), Season (H. S. Hussein and Bollinger, 2005), Type of product/ area sampled (Hussein, 2007), Presence of super shedders (Omisakin et al., 2003)

The high variability and the various factors that influence the presence and survival of *E.coli* O157:H7 make it hard to accurately generalize results. In the Midwest itself rates of 0.3% to 12.9% have been recorded in just three processing houses (Barkocy-Gallagher et al., 2003).

2.5 *Salmonella* spp., in cattle

2.5.1   Introduction

Cattle are reservoirs for multiple *Salmonella* species (Wells et al., 2001). *Salmonella* has been frequently isolated from farms but not all strains have been implicated in human disease (Crump et al., 2002; Davis et al., 2003). *Salmonella* has around 2500 subspecies and each one is capable of infecting different animals making it a zoonotic disease agent; *S. Dublin* causing symptoms in cattle, *S.typhi* in humans (Poppe, 2011). As a foodborne disease *Salmonella* accounts for around 1,000,000 infections per year (Scallan et al., 2011) thereby making it an important foodborne pathogen. Although originally associated with poultry *Salmonella* outbreaks have been traced to a variety of foods like leafy greens, peanut butter, and meat (Scallan et al., 2011). Manure has been shown to be a suitable vehicle for

Salmonella transmission to leafy greens and since organic systems rely heavily on manure as a soil amendment control of this pathogen is even more important (Franz et al., 2005; Holley et al., 2007; Jacobsen and Bech, 2012; Maciorowski et al., 2007). Multiple sources of *Salmonella* spp., are found on farms with feed, wildlife, fecal matter, and infected cattle being the major sources. Cull dairy cows have been found to have high levels of *Salmonella* shed in their feces with at least 66% testing as positive (Wells et al., 2001), which makes it evident that steps should be taken to reduce contamination as once established pathogens are hard to eliminate from the environment; making it wise to keep levels minimal to reduce the risk of outbreaks (Fossler et al., 2005b).

2.5.2   Sources of *Salmonella* spp.

2.5.2.1 Feed and Pasture

Cattle diets include a lot of forage material like grass, hay, and rye. The diet may be supplemented with feed "concentrates" which include grains like corn, and soy. Like every living organism cattle need to eat to grow and in the beef industry growth and maintenance of mass is very important and can be achieved by using high quality feeds. Feed is therefore a component of production to which cattle have high exposure to and feedstuff has been regularly found to be contaminated with *Salmonella* species (Dargatz et al., 2005). Cattle feed in the Pacific Northwestern USA was found to have a prevalence of 9.8% with dairies being more likely to be contaminated as dairy cows have a higher feed requirements (Krytenburg et al., 1998).

As stated earlier organic farmers frequently amend soil using manure which is a an effective vehicle for *Salmonella* transmission (Franz et al., 2005; Holley et al., 2007) as, survival in soil can result in contaminated vegetative matter (Jacobsen and Bech, 2012). Cattle on pasture may therefore be exposed to *Salmonella* present in the soil via deposits of contaminated fecal matter or manure. Organic systems dictate that cattle have a minimum of 120 days on pasture (Coffey and Baier, 2012) this may present an increased risk of infection and colonization of *Salmonella* in the lower guts of cattle; warranting some aspect of control. Detection of the presence of *Salmonella* spp., in feeds is not an indication of presence in cattle and so it is necessary to test the cattle fecal matter for *Salmonella* and then compare the isolates using PFGE or other serological data (James C Carlson et al., 2015a).

Not all isolates are responsible for outbreaks of disease in humans (Krytenburg et al., 1998) but some, for example, *S. thyphimurium* is frequently isolated in feed and is often implicated in human outbreaks (Davis et al., 2003). *Salmonella* is notorious for its' ability to survive in dry environments and dry food matrices with water activities as low as 0.7 have been found to harbor *Salmonella* thereby making feed an important source of contamination on farms (Juven et al., 1984; Margas et al., 2014) especially since cattle have repeated exposure to feed at all stages of life.

2.5.2.2 Water

Oral inoculation of *Salmonella* is possible which makes contaminated water troughs a potential source of infection (Poppe, 2011; Rodriguez-Rivera et al., 2014). The presence of organic matter such as feces, cud or even feed may enhance the survival of *Salmonella* spp.,

in water trough and thorough cleaning has been shown to be effective in controlling and even eliminating *Salmonella* from water troughs (LeJeune et al., 2001). *Salmonella* has been shown to be carried by waterways and streams running through pasture. It has been observed that *Salmonella* can be leached out of fecal matter or manure be dissipated via waterways (Fangueiro et al., 2014; Forslund et al., 2011; Mostofa Amin et al., 2013). Survival is affected by season with spring and summer having a greater chance and length of survival in water as seen by LeJeune et al, 2001. *Salmonella* however has a much lower survivability than *E.coli* in water and is therefore seen as less of a problem via the water borne route especially if an adequately sanitary steps are taken and organic load of water troughs is kept at minimal levels (LeJeune et al., 2001).

2.5.2.3 Manure

Fecal shedding of *Salmonella* spp., has been frequently reported (Fossler et al., 2005a, 2005b; Maciorowski et al., 2007; Van Donkersgoed et al., 1999). Manure in the form of compost is added to organic soils in order to boost fertility (Coffey and Baier, 2012) and when improperly composted can be a source of contamination in system (Sinton et al., 2007; Temple et al., 1980). It has been shown that *Salmonella* present in the soil can be transferred to vegetation and animals or humans consuming them (Hsi et al., 2015; Scallan et al., 2011). The maximum time *Salmonella* can subsist in soil amended with manure is highly variable and there have been reports that range from 25 days (Dorn and Schleiff, 1997) to over eight weeks at 10^3 CFU/g (Temple et al., 1980) with variations seen over seasons denoting a temperature-humidity relationship as well (Maciorowski et al., 2007). *Salmonella* shedding in feces is seen

to peak in the warmer months (Barkocy-Gallagher et al., 2003) which is also when cattle are likely to be out to pasture thereby increasing the risk of transmission. Manure is therefore a viable source of *Salmonella* on the farm especially if proper composting is not achieved which suggests that one method of control is to ensure and enforce proper composting of fecal matter on organic farms. Organic matter deposited by cows on pasture also contribute towards the spread of *Salmonella* and so sick cows should be isolated wherever possible, this is not always possible however as cows are mostly carriers of strains that cause disease in humans. Isolation of sick animals however would prevent other animals from getting sick and is a recommended good practice (Coffey and Baier, 2012).

2.5.2.4 Wildlife

Wildlife especially of avian origin represent a huge mobile reservoir of *Salmonella* (James C. Carlson et al., 2015; Hilbert et al., 2012; Tizard, 2004) with starlings being frequently implicated in the spread of *Salmonella* from farm to farm. There is some conflict however as to the significance of starlings transmitting disease as concluded by Gaukler et al., 2009. The study by Carlson et al., 2015 found that 17% of external wash starling samples were contaminated with *Salmonella* and all the serotypes isolated were isolated from the farm as well thereby laying out a possibility of the birds as a source of contamination. Other studies reported that different species of birds have different rates of *Salmonella* prevalence and so avian sources should not be written off (Refsum et al., 2002). As a zoonotic microbe *Salmonella* vectors also include insects like flies, beetles and ticks (Wales et al., 2010). Insects are much smaller in size than birds and may therefore prove harder to control. The risk

associated with insects being a source of contamination in the farm environment has yet to be properly studied and quantified. Vertical transmission of *Salmonella* is also possible with positive dams passing on the organism to their calves (Hanson et al., 2015) which may indicate that pregnant cows be monitored and treated to ensure that their respective calves are *Salmonella* free. Insects (especially flies), birds and infected cows are therefore thought to be the major animal sources of *Salmonella* on farms.

2.5.3   Prevalence of *Salmonella* spp.

When dealing with living systems there is a lot of variability as these systems are dynamic. *Salmonella* follows this pattern and rates of occurrence vary with respect to: Season (Barkocy-Gallagher et al., 2003; Fossler et al., 2005b), Disease history of animal (Cummings et al., 2009; Fossler et al., 2005b; Nielsen and Dohoo, 2012), Geographical location (Fossler et al., 2005b), Wildlife interactions (Carlson et al., 2015b; Refsum et al., 2002), Herd size (Fossler et al., 2005b), Status of pregnant dam (Hanson et al., 2015).

Salmonella prevalence in feed is around 9.8% with slight differences between feed type; ±3%, with dry forage at 8.5%, wet forage at 10.9% (Krytenburg et al., 1998). Arnold et al.,2015, found that in the UK *Salmonella* prevalence amongst outdoor cattle was higher than indoor cattle; 38.7% versus 22.1%. They however attributed this difference to the fact that one large dairy that had a high prevalence rate and not to management type (Arnold et al., 2015). In Midwestern USA *Salmonella* spp., was isolated from 4.4% fecal, 71.0% hide and 12.7% carcass samples (Barkocy-Gallagher et al., 2003). These were obtained from cattle at a processing facility and so may not reflect on farm rates. There was however seasonal

variation observed. The effect of geography or location is also made evident as different studies based in various countries had different isolation rates; Ethiopia 7.6% (Muluneh and Kibret, 2015), Southern Brazil 0.93% (Loiko et al., 2016). Cows that have been sick or currently sick (diarrheal symptoms, mastitis) show a higher prevalence in fecal shedding than healthy cattle along with an increased duration of shedding (Cummings et al., 2009), further advocating the need to isolate visibly sick animals.

The type of sample taken: hide, fecal or meat also shows variations in prevalence with hide showing the higher rates followed by fecal and carcass (Barkocy-Gallagher et al., 2003; Brichta-Harhay et al., 2008). These studies however looked at cattle in processing facilities and they attributed the stress of transport (Barham et al., 2002). Fortunately post-harvest treatments are effective at reducing loads and prevalence of *Salmonella,* though variable on carcasses is much lower in all cases (Bacon et al., 2002; Barkocy-Gallagher et al., 2003) with prevalence rates dropping to 1.3-0% in facilities in the United States of America. The nature and extent of processing however has an effect on the prevalence rates in final beef products which are again highly variable.

2.6 Contamination events at harvest

It has been shown that although low levels of *Salmonella* and *E.coli* O157:H7 are present during the farm and rearing phase, these levels tend go up especially on hides during or after transport to a slaughter facility (Barham et al., 2002a; Beach et al., 2002).This is possibly due to the stress of transport, and the higher density of cattle present during transport. It has been noted that after transport and at slaughter facilities fecal shedding

appears reduced but prevalence of both pathogens on the hide increase (Arthur et al., 2011; Beach et al., 2002; Narváez-Bravo et al., 2013). Visible dirtiness of hides was found to not be an indicator of the presence of absence of pathogens but it was noted that visibly dirty hides had a higher microbial load and therefore were less affected by post-harvest interventions than visibly clean surfaces (Hauge et al., 2015).

It is thought that pathogens are transferred to the carcass at the de-hiding and evisceration stages (Martínez-chávez et al., 2015; Nastasijevic et al., 2009). Leakage of ruminal fluid during evisceration is thought to be a source of contamination. Current practices however are effective at reducing loads and include practices such as hot water washing, steam vacuuming, washing with organic acids and steam pasteurization (Arthur et al., 2002). It is important to note that effectiveness of such interventions depends on initial microbial loads and so those carcass/cattle with higher microbial loads may still contain pathogens (Hauge et al., 2015).

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CHAPTER 3: A MINI-REVIEW OF PATHOGENS ASSOCIATED WITH GROUND BEEF AND

FERMENTED SAUSAGE PRODUCTS; INTERVENTIONS APPLIED TO ENSURE FOOD SAFETY

Introduction

Beef is a common source of protein for the masses. Undercooked beef and derived products may however be sources of foodborne pathogens (Crump et al., 2002; H. S. Hussein and Bollinger, 2005). It is estimated that from 1998-2008, beef and other meat products were responsible for 22% of the foodborne outbreaks with beef contributing to 6% of these (Painter et al., 2013). Painter etal., 2013, also found that deaths related to illnesses caused by meat and poultry products were the highest at 43% and the same class of products was frequently implicated in bacterial related outbreaks for the same time period. Major bacterial pathogens associated with meat and derived products are; pathogenic *Escherichia coli*, *Salmonella* spp., *Campylobacter jejuni* (Fox et al., 2008; Schlundt, 2002). Outbreak data from 2004-2010 shows that mainly STEC and *Salmonella* spp., have been attributed to beef in particular (Crowe et al., 2015). Literature indicates that there is further scope to improve production and processing of meat products.

Ground beef is notorious for being a vehicle in outbreaks (H. H. S. Hussein and Bollinger, 2005; Rangel et al., 2005) and pathogens such as pathogenic *Escherichia coli*, *Salmonella* and *Campylobacter* species have been isolated in retail beef samples across the United State of America (H. S. Hussein and Bollinger, 2005; LeJeune and Christie, 2004; Samadpour et al., 2006). Although found at low prevalence the presence of pathogens in meat does indicate that there is a risk of contracting a foodborne illness by consuming

undercooked meat. It was should also be noted that different strains might have different thermal resistances for example non-O157 *E. coli* was found to have higher thermal resistance than E.coli O157:H7 in marinated steaks (Pokharel et al., 2016). Different pathogens have different occurrence rates depending on the processing factors involved for example in a study conducted to determine *Salmonella* and *Campylobacter* levels it was found that campylobacter was more likely to be isolated from whole muscle cuts (17.4%) rather than ground beef (9.3%) (Vipham et al., 2012).

Several interventions now exist in order to ensure that pathogens are killed. These processes also have an added advantage as they kill or restrict the growth of spoilage microbes as well and so can confer shelf-life extension (Ercolini et al., 2011; Morey et al., 2014). Modified atmosphere packaging is commonly used to control the growth of microbes (Ammor et al., 2009)   and this when combined with other technologies such as irradiation and antimicrobial films can be used to control the growth of pathogens (Gunes et al., 2011; Kudra et al., 2013). Novel uses of compounds like chitosan to make edible or biodegradable packaging are also emerging (Beverlya et al., 2008; Hugo and Hugo, 2015; Ye et al., 2008).

The many varieties of products available along with the differences in packaging, storage, pathogens, and microflora involved make comparison of meat products complex and highly variable. This review will aim to look at pathogens in ground beef and, sausage which are considered to be ready to eat, followed by potential interventions that could be applied to ensure safety.

Ground beef

Ground beef is made from trimmings and sometimes offal that would otherwise be sold directly to consumers; lymph nodes which are sometimes present in ground beef are known to be a source of *Salmonella* spp., and can potentially cause contamination (Koohmaraie et al., 2012; Li et al., 2015). Deep tissue lymph nodes which are found in the cuts used to make ground beef have been known to contain *Salmonella* spp. In a study conducted by Gragg et al.,2013, it was observed that *Salmonella* spp., was present in the subiliac lymph nodes of 11.8% of feedlot cattle and 0.65% of cull cattle. In cattle with positive lymph samples, 67% had levels ranging from 0.1 to 1.8 log CFU/g and, 33% had levels 1.9 to greater than 3.8 log CFU/g (Gragg et al., 2013). Koohmaraie et al., 2012, also found similar results for *Salmonella* prevalence in lymph nodes of dairy cows with 18% of samples testing positive (Koohmaraie et al., 2012).This is important as carcass intervention methods do not penetrate into tissue meaning the *Salmonella* population is not reduced by interventions applied. From 1973 to 2011 around 1965 outbreaks of *Salmonella* were reported; beef was linked to 96 out of which ground beef was further implicated in 23% whilst 18% were unknown (Laufer et al., 2015). Prevalence rates of *Salmonella* in ground beef are found to range from 0.55 to 3.83% (Hill et al., 2011; Samadpour et al., 2006; Vipham et al., 2012). From 1992-2002 there were 350 outbreaks of *E.coli* O157:H7 and a 183 of these were foodborne. Of the foodborne related outbreaks ground beef was the carrier in 41% of cases (Rangel et al., 2005). Outbreaks of *Escherichia coli* O157:H7 are frequently associated with ground beef; it is in fact, colloquially known as the "hamburger disease"(Callaway et al., 2003). *E.coli* O157:H7 was declared an adulterant in ground beef in 1994 and regulations are laid out in 9 CFR Chapter 3 (*Federal*

Register: Beef Products Contaminated with Escherichia Coli 0157:H7, 1999). Beef that is found contaminated with *E.coli* O157:H7 must be directed towards processes that will ensure kill or be destroyed. The FSIS cannot however monitor every single ground beef package produced but does sample on a risk based method with higher production rates leading to an increased sampling frequency. Prevalence rates at retails levels have been found to be 0 to 0.34%, however presence of total STEC is found to be much higher ranging from 5.5% to 35% (Hill et al., 2011; Samadpour et al., 2006; Svoboda et al., 2013). *Salmonella* spp., and *E. coli* O157:H7 are major foodborne pathogens associated with ground beef due to the high number of outbreaks. Although no outbreaks linked to *C. jejuni* and *L. monocytogenes* have been recorded studies have indicated that they have been present in ground beef samples obtained from retail stores (Samadpour et al., 2006; Vipham et al., 2012). *C. jejuni* was found in 7.35% of ground beef obtained from retailed stores (Vipham et al., 2012) and *L. monocytogenes* was isolated from 3.5% of samples of ground beef (Samadpour et al., 2006).

Due to the frequent occurrence of outbreaks of *E. coli* O157:H7 and *Salmonella* spp., in ground beef these two will be focused on. The USDA-FSIS declared *E. coli* O157:H7 as an adulterant in beef in 1994 and is detailed out in 9 CFR Chapter 3 (*Federal Register: Beef Products Contaminated with Escherichia Coli 0157:H7*, 1999) but studies have detected low levels of the pathogen in ground beef (Hill et al., 2011; Samadpour et al., 2006; Vipham et al., 2012) around 3.83% at maximum. Although the methods used did not detect any organisms, further enrichment indicated low numbers of the cells were present. *E. coli* O157:H7 has been shown to replicate in ground beef even at temperatures as low as 6°C (Tamplin et al., 2005). In a study by Tamplin et al., 2002, the exponential growth rate for E.coli O157:H7 was 0.019

log CFU/hr with a maximum population density of 5.09 log CFU/g (Tamplin, 2002). This coupled with the low infectious dose of approximately 50 cells (Lim et al., 2010) make the presence of even a small number of cells a risk especially if the product is subject to temperature abuse. *Salmonella* spp., can also grow at temperatures around 10°C in ground beef (Fujikawa et al., 2015; Juneja et al., 2009). The specific growth rate for *Salmonella* at 10°C is less than 0.2 log CFU/g/hr (Juneja et al., 2009).The infectious dose of *Salmonella* spp., varies with strain but temperature abuse can lead to an increase in numbers enough to cause sickness as above 20°C the growth rate is more than 0.4 log CFU/g/hr with a peak of 1log CFU/g/hr at 40°C (Juneja et al., 2009). Currently data shows a downward trend of STEC related outbreaks in all foods that are monitored by the USDA-FSIS; from 20 outbreaks in 2009 to over 5 in 2012, (Robertson et al., 2016) indicating that efforts to reduce STEC might be paying off.

Seasonal variation is seen in outbreaks with the summer months seeing a greater proportion of outbreaks (Rangel et al., 2005; Robertson et al., 2016; Williams et al., 2010). Rangel et al.,2005, reported that out of the outbreaks associated with *E.coli* O157:H7 in ground beef from 1982-2002, 71% were during the summer months. 41% of all outbreaks of STEC from 2007 to 2012 were also summertime outbreaks with no apparent seasonality pattern for *Salmonella* (Robertson et al., 2016).

Frozen storage effects both *Salmonella* spp., and *E.coli* O 157:H7 and can cause a reduction of upto 0.7 log CFU/g when held at -22°C (Manios and Skandamis, 2015). Thawing conditions also influence levels with thawing on the kitchen counter (20°C for 12 hours) witnessing increased levels of 0.9 log CFU/g but no significant was evident with microwave

thawing or thawing at 4°C (Manios and Skandamis, 2015). The study by Manios et al., 2015, also examined two cooking methods oven-broiler and pan grilling at 71°C and 60°C to represent cooked and undercooked meat. The oven broiler at 71°C was more efficient at reducing the levels to below the methods detection limits (0.7 log CFU/g) which was not seen in the pan-grill method even at 71°C which had a 4 log reduction even in the best case scenario (thawing at 4°C, and cooking at 71°C). This is explained by the method of heat conduction in the two systems; in the pan-grill only conduction, but, in the oven broiler heat is transmitted by conduction and convention exposing pathogens to greater heat levels and therefore achieving a higher kill (Manios and Skandamis, 2015). Double sided broiling was also found to be more efficient at reducing E.coli O157:H7 levels in ground beef patties than single sided broiling (5.7 log CFU/g log reduction versus 1.3 log CFU/g reduction) when cooked to an internal temperature of 71.2°C and this was attributed to the greater and faster penetration of heat in the double sided system (D'Sa et al., 2000).

The occurrence of outbreaks indicates that more could be done in areas like retail distribution and consumer education. Temperature abuse during distribution and transport plays a role in the reproduction of organisms and transmission of disease (Buncic et al., 2014; Currie et al., 2007) indicating that better control is needed. Consumers should also be educated about the risks of consuming undercooked meat and the usage of thermometers to monitor temperature (Currie et al., 2007; Vogt and Dippold, 2005) to reduce cases of foodborne illnesses.

Fermented Sausages

Fermented foods are generally used to extend shelf-life generally by action of micro-organisms and their biochemical processes which are inhibitory to other microorganisms. The major pathogens associated with fermented sausages are *Salmonella* spp., VTEC, *Staphylococcus aureus* and to a lesser extent *Listeria monocytogenes* (Adams and Mitchell, 2002; Adams and Nicolaides, 1997; Farber et al., 1993). The fermentation process for meats involves an inoculation step, a curing and ripening step and sometimes a smoking step. Several studies have been conducted on to determine the impacts of these conditions on the major foodborne associated pathogenic strains viz *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes*. Fermented sausages come under the ready to eat (RTE) category of foods.

RTE foods that contain *Listeria monocytogenes* are considered adulterated and producers that produce such foods need to address such risks in their HACCP plan, Sanitation SOP's or other prerequisite programs in order to comply with 9 CFR part 430.

Several hurdles are present in fermented foods such as presence of nitrates, sodium chloride, lactic acid, lactic acid bacteria, phenols, heat and low water activity (Adams and Mitchell, 2002). The FSIS requires processors achieve a 5log reduction in viable numbers *E. coli* O157:H7 which resulted in many studies being carried out to determine the effective of the fermenting process (Getty et al., 2000).

A challenge study using multiple strains of *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* showed that the fermentation process was sufficient at reducing loads of pathogens and that temperature, time and pH were significant factors that affected

viability of pathogens (Hwang et al., 2009). In the study conducted by Hwang et al., 2009, on soudjouk-style sausages, the reduction after fermentation, drying and storage at 4°C, 21°C and 30°C was monitored. The change in pH along with the change in water activity and their effect on pathogen growth was also examined. Hwang et al., 2009, concluded that during fermentation each pathogen had a different reduction level *E.coli* O157:H7 0.9-1 log CFU/g, *Salmonella* spp., 3 log CFU/g and *Listeria monocytogenes* 0.5 log CFU/g at a pH of 4.6. Further reduction of pathogens was seen during the drying stage (pH 4.6 and water activity 0.85); *E.coli* O157:H7 3.5 log CFU/g, *Salmonella* spp., 2 log CFU/g and *Listeria monocytogenes* 0.5 log CFU/g. Higher storage temperatures were also associated with greater reduction in loads of all the pathogens. Hwang et al.,2009, determined that pH, water activity, and storage temperature were important factors. However, after analyzing forty-four independent studies of various types of sausages studies McQuestin et al.,2009, determined that temperature was the single most important factor that determined survivability of pathogens as temperature of fermentation accounted for 61% of the variability seen in the levels of various *E.coli* strains used (McQuestin et al., 2009) with higher temperatures being associated with increased reduction. Temperature of fermentation was also found to explain 60% of variance seen in a separate study conducted by Mataragas et el., 2015, on various types of Italian sausage (Mataragas et al., 2015b).

The fermentation process of dried sausages occurs with the help of lactic acid bacteria (LAB) and these are known to have inhibitory effects on pathogens as they reduce the pH via the production of lactic acid, produce anti-microbial compounds (nisin, hydrogen peroxide) and also outcompete pathogens for resources (Adams and Nicolaides, 1997). Reduction of

pH is important as they enable organic acids like lactic acids and ethanoic acid to penetrate cell membranes and then dissociate in the relatively higher pH of the bacterial cells; where they cause cell death. LAB also produce hydrogen peroxide which can inhibit the growth of S. aureus (Adams and Nicolaides, 1997). Bacteriocins like nisin are also produced by LAB and they are known to have inhibitory effects on microbes like *Salmonella* spp., and *E.coli* (Adams and Nicolaides, 1997). LAB are generally inoculated at levels of 7 log CFU/g and there is an increase of 2-5 log cycles of their population during the fermentation process (Hwang et al., 2009; Yusof et al., 1993). It should be noted however that *Listeria monocytogenes* are more resilient than *Salmonella* spp., when it comes to surviving the fermentation process (Mataragas et al., 2015a).

Most studies agree that the fermentation process is adequate when it comes to reducing levels of pathogens provided the minimum degree hour requirements, pH, and use of appropriate starter cultures are used. Some processes such as those used for Italian salami may not however be adequate and so additional steps may be needed in order to ensure consumers obtain a safe product such as; raw product specifications, longer curing times and cooking or heating (Dalzini et al., 2015; Nightingale et al., 2006). Dalzini et al., 2015, found that the processing methods used for low fat salami were unsatisfactory at reducing pathogen levels as laid out by the FSIS as reduction levels obtained were 2.5 log CFU/g *E.coli*, 1.65 CFU/g *S. Thyphimurium*, and 0.5 log CFU/g *Listeria monocytogenes* when initial inoculation levels were 5log CFU/g (Dalzini et al., 2015). This trend fits in with data available with the last major outbreak in the USA being in 1994 and involving salami as reported by the Mortality and Morbidity Weekly Report (Anonymous, 1995). Other outbreaks have

occurred and with different types of sausages but these were due to improper hygiene or storage, after processing or during slicing (Gormley et al., 2010). In the study conducted by Gormley et al., 2010, it was found that 72.2% of the products sliced on request in store and had greater than 100 CFU/g of *E. coli*. An outbreak of Listeria monocytogenes in a hospital in Germany in that went on from 2006-2008 was traced back to a scalded sausage product and isolates matching the outbreak strain was obtained from both the production environment and the kitchen that unpacked and served the sausages (Winter et al., 2009). The outbreak further reinforces how postprocessing contamination is the major cause of outbreaks related to fermented sausage and ready-to-eat food products.

Interventions

Post-harvest interventions

Post-harvest interventions are applied after slaughter of cattle. They are seen to be much more effective and economical than on farm interventions as cattle are often contaminated at the processing facility and, these interventions target groups of pathogens rather over specific ones (Koohmaraie et al., 2005). At the processing level *Escherichia coli* O157:H7 and *Salmonella* spp., are considered to be the major pathogens of concern (Koohmaraie et al., 2007, 2005; Martínez-Chávez et al., 2015) and are therefore the major focus of intervention methods.

Hide is considered to be a significant source of pathogens especially in large facilities where cattle can co-mingle for extended periods (Arthur et al., 2011, 2007; Bacon et al., 2002; Brichta-Harhay et al., 2008; Narváez-Bravo et al., 2013). Arthur et al., 2001, found STEC on

43% of cattle hide in the spring and to a high of 78% in the fall. *Salmonella* was found in 71% of hide samples of cattle at a processing facility (Barkocy-Gallagher et al., 2003). Koohmaraie et al.,2005, found a much higher prevalence of *Salmonella* on hide with ninety six out of a hundred cows testing positive. Post- harvest interventions therefore see decontamination of hide by: dehairing, or chemical washing (Cetylpyridinium chloride, sodium hydroxide, phosphoric acid) (Koohmaraie et al., 2005) as useful means of reducing microbial loads on hide and therefore carcass and meat. Hide washing cabinets are also available and these are ideal for large processors as they are automated. In a study conducted by Arthur et al.,2007, pre-intervention hides prevalence of *E.coli* O 157:H7 on hides was 97.6% but after washing with water and chlorine (100-200 ppm) was reduced to 89.6%. The study also examined the effect of the washing intervention on *Salmonella* prevalence which was reduced to 68.8% from 94.8% (Arthur et al., 2007). The dehairing of hide was also shown to reduce the prevalence of E.coli O157:H7 in carcass and hide samples; from 88% to 67% on hide and from 50% to 1% on carcass (Nou et al., 2003). The study by Nou et al., 2003, effectively showed that dehaired hides effectively lowered the prevalence on the carcasses and so is a viable option for reduction of prevalence of pathogens.

Carcass interventions are also applied and these include measures like steam vacuuming, application of organic acids (acetic acid, lactic acid) or a combination of several techniques (Koohmaraie et al., 2005; Zhilyaev et al., 2017). Zhilyaev et al.,2017, concluded that the following reductions (log CFU/cm^2) could be made in *E.coli* populations: acetic acid - 1.44, lactic acid -2.07, steam vacuum – 3.09 and, hot water – 1.90. Dorsa et al., worked on

Steam vacuuming of carcass as an intervention and found that a reduction of 4 log CFU/cm^2

when used in conjunction with a warm water (30°C) and high pressure (125 psi) wash (Dorsa

et al., 1996).

The interventions applied at post-harvest are thought to be effective in reducing the

load of microbes in the system and not eliminate them completely therefore, caution still has

be exercised when dealing with raw beef (Zhilyaev et al., 2017).

Processing Level Interventions

Once slaughtered the carcass is then fabricated and shipped off for further processing. Some

applications use "hot" meat whilst others use a carcass that has been chilled for around 24-

36 hours. Beef can be cut into smaller units and then sold as raw beef, ground beef, and other

processed products. Processors can use a variety of techniques to ensure that their products

are safe to each such as: Organic Acids and Natural Antimicrobials, Antimicrobial Compounds,

Fermentation, Heat/Cooking, and Packaging.

i.   Organic Acids and Natural Antimicrobials

These have gained popularity especially with the consumer movement towards more

natural foods (Fox et al., 2008). Organic acids used in meat products include acetic acid, citric

acid, lactic acid, propionic acid, malic acid, succinic acid and tartaric acid (E. Mani-López et al.,

2012). They are weak acids and have been found to be more effective at lower pH levels

especially below their pKa. Organic acids and their salts are Generally Recognized as Safe

(GRAS) substances which have no daily limit for human consumption (E. Mani-López et al.,
2012) and, can be easily incorporated in a product. A major drawback is they produce off-
flavors in products even though they effectively suppress growth of pathogens (Morey et al.,
2014; Smulders and Greer, 1998). In the study conducted by Morey et al.,2014, it was found
that treatments of frankfurter batters with 2% Sodium Lactate or, 2% Potassium Lactate or
a combination of 2% Sodium Lactate with 0.25% sodium diacetate were able to restrict the
growth of *Listeria monocytogenes* for over 8 weeks at 4°C however, sodium citrate at 2% did
not inhibit growth and there was a 2log CFU/frank increase which was similar to the control.
The sensory aspects were not severely affected by Sodium lactate, sodium citrate or
potassium lactate but the combination of sodium lactate and sodium diacetate adversely
affected sensory aspects with franks being unacceptable after 1 week of storage. The
effectiveness of sodium lactate at low levels was examined by Ye et al.,2008, who
incorporated sodium lactate in film at a rate of 0.01 g/cm^2. The film sodium lactate was then
placed on ham which was inoculated with *Listeria monocytogenes* and stored at 4°C and a
reduction of 1.2 log CFU/cm^2 was observed for up to ten weeks (Ye et al., 2008). A major
concern with organic acids is that *E.coli* O157:H7 and *Salmonella* spp., can develop acid
tolerance thereby limiting the usage of organic acids and should therefore be supplemented
with other treatments (Smulders and Greer, 1998). The mechanism by which organic acids
such as lactic acid and ethanoic acid are well studied. Organic acids are weak acids that do
not dissociate unless the pH is above their pK$_a$. At a low pH organic acids are uncharged and
can cross the cell membranes. Once inside the cell cytoplasm the pH is much higher than the
pK$_a$ and, this causes the organic acid to dissociate. The organism now has to spend energy to

pump out the proton in order to maintain equilibrium and there is a toxic buildup of the anion in the cytoplasm. Organic acids with lower pK$_a$'s therefore make stronger antimicrobials than those with higher pK$_a$'s (Adams and Nicolaides, 1997).

Natural antimicrobials such as essential oils from plants and bacteriocins are also being explored for their use in control of foodborne pathogens (Bajpai et al., 2012). Essential oils have been shown to have inhibitory effects against *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* (Boskovic et al., 2015; Burt, 2004; Hayouni et al., 2008; Khaleque et al., 2016). The mechanisms by which essential oils act is currently under study. Boskovic et al., 2015, examined the minimum inhibitory concentration (MIC) of Thyme and Oregano extracts which, principally contain thymol and carvacrol on strains of *Salmonella Enteritidis*; *Salmonella Thyphimurium*; *Staphylococcus aureus*; and *Escherichia coli*. Oregano and Thyme extract had a MIC of 320µg/ml for *Salmonella Enteritidis*, and *Escherichia coli*. *Staphylococcus aureus* was more resistant and a concentration of at least 640 µg/ml was needed to exhibit inhibition. *Salmonella Thyphimurium* was susceptible to Oregano extract as it had an MIC of 160 µg/ml. The mechanism of action of these two essential oils to thought to be membrane degradation which allows for leakage of ions and increased cell membrane permeability (Lambert et al., 2001). It was determined by Khanleque et al.,2016, that cinnamon essential oil, when used at levels of 5% was found to cause a 3.5-4 log CFU/g reduction of Listeria monocytogenes at 0°C and 4°C (Khaleque et al., 2016). Hayouni et al.,2008, examined the effects of sage and pink pepper essential oils on *S. anatum* and *S. enteritidis* in minced beef. Both oils showed a 3log CFU/g when used separately at levels of

around 3% although when examined by a the alterations made to the product was deemed unacceptable in uninoculated samples evaluated by the sensory panel (Hayouni et al., 2008). This shows that although essential oils are effective they significantly alter sensory aspects and so need to be used in applications where their flavors are masked or appreciated (Burt, 2004). Tayel et al., 2012, used pomegranate peel extract, cinnamon bark extract and lemon grass extract at their MIC values of 250µg/ml, 350µg/ml, and 550 µg/ml respectively. The extracts were applied separately or together at the MIC concentrations to steaks that were inoculated with 7 Log CFU/ml of *Salmonella Typhimurium* and *Staphylococcus aureus*. It was found that a combination of all three extracts was effective at reducing populations of both pathogens by at least 5 log CFU/g when stored at 4°C for 7 days. The sensory panel also rated this treatment the highest for overall acceptability on the 7th day (Tayel et al., 2012). The study by Tayel et al., 2008, shows that if used in the right kind of application essential oils can be used at effective concentrations in food products.

Bacteriocins have also been used as antimicrobials with nisin being widely used. Nisin was found to have an inhibitory effect on *Listeria monocytogenes* when used at 1000 IU/g though growth is seen to resume after 6-7 days of storage at refrigeration temperatures and so was not seen as a reliable standalone ingredient to control the growth of *Listeria monocytogenes* (Solomakos et al., 2008). Govaris et al.,2010, used nisin at levels of 500 and 1000IU/g and observed that there was no effect on *Salmonella Enteritidis* when it was used alone but, when combined with oregano extract at a level of 0.9% oregano extract and 500 IU/g nisin there was a 4log CFU/g within 48 hours. The limited activity of nisin and its

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synergistic effect when applied in conjunction with natural antimicrobials is currently being looked at as a way to reduce the levels of essential oils used and therefore prevent the alteration of off-flavors (Govaris et al., 2010).

Natural antimicrobials can be supplemented by using new and novel techniques such as High Pressure Processing (HPP) in order to achieve maximum inactivation of pathogens with minimum impact on flavor (Aymerich et al., 2005). Aymerich et al.,2005, showed that a combination of HPP and bacteriocins at 2000AU/cm^2 was able to reduce 4log of *Listeria monocytogenes* to less than 0.5log CFU/g and maintained its bactericidal action for 60 days when stored at 6°C. The combination of several components like essential oils, bacteriocins and novel techniques like HPP can therefore be used to reduce and suppress the levels of pathogens in food.

ii.   Antimicrobial Compounds

Peroxyacetic acid, sodium chlorite, sulfites, phenols, and nitrates are examples of some chemical antimicrobial compounds. Peroxyacetic acid and acidified sodium chlorite have been found to reduce microbial loads in surfaces of loin when used at 205 ppm in marination applications where it reduced the level of *E.coli* O157:H7 and *Salmonella* counts by 1 log CFU/g (Ulbrich et al., 2015). Sodium Sulfite is also used as a chemical antimicrobial although it is not as effective as the other chemicals in use currently (Sultana et al., 2014). The anti-microbial activity of a 10μg/ml aqueous sodium sulfite solution was shown to have a zone of inhibition of at least 30mm against several pathogens like *E. coli*, *Klebsiella* spp.,

Pseudomonas spp., *Salmonella* spp., *Staphylococcus* spp., *Vibrio* spp., *Listeria* spp., and *Bacillus* spp., (Sultana et al., 2014). Sodium sulfite has a disadvantage in that members of the population exhibit sensitivity towards the compound with various adverse reactions. Phenols though used to give a smoky flavor also exhibit antimicrobial activity and are used especially in sausages and fermented products (Hwang et al., 2009). Alakomi et al.,2007, used berry extracts on *Salmonella* strains and found that they had an increased sensitivity to novobiocin; the zone of inhibition was increased to 11mm instead of 14mm. Phenols have been shown to destabilize the outer membranes of Gram-negative bacteria thereby weakening them and causing an increased susceptibility to antibiotics, heat and other adverse conditions (Alakomi et al., 2007).

The negative connotations associated with chemical or preservative amongst consumers has now led the Food Industry to migrate away from using them in products (Fox et al., 2008). Industry is now actively looking at and evaluating the use of "more" natural products in order to meet consumer expectation without greatly sacrificing safety (Hugo and Hugo, 2015).

iii.   Fermentation

As stated earlier fermentation is one of the methods by which food is preserved (Adams and Mitchell, 2002).Fermented foods have wide acceptability by consumers making the control of the process very important. Several "hurdles" such as low water activity, low pH, heat and presence of Lactic Acid Bacteria (LAB) (Adams and Nicolaides, 1997) are present

in the fermentation process. Most fermented meat products utilize cultures of LAB that may be hetero or homo- fermentative. Heterofermentative produce mixtures of organic acids whilst homofermentative generally produce one product (lactic acid). Heterofermentative strains produce ethanoic and lactic acid which have good synergy and are effective at destroying most pathogens (Adams and Nicolaides, 1997). Temperature at which fermentation is carried out is also important; higher temperature and longer times lead to greater inactivation of pathogens (Hwang et al., 2009). Nickelson et al.,1996, also demonstrated that lower temperatures with longer fermentation times or higher temperatures with shorter times were associated with greater reduction in numbers of *E.coli* O157:H7; fermenting at 32.2 °C (pH 4.6) for 6 days caused a 5 log reduction of *E. coli* O157:H7 whereas at 37.8 °C this reduction was seen in 4 days (Nickelson II et al., 1996).

The LAB strains used to inoculate meat products produce several chemicals that are known to suppress growth of pathogens like lactic acid, bacteriocins, hydrogen peroxide, and compete with pathogens and spoilage organisms for resources (Adams and Nicolaides, 1997). The role of fermentation has been discussed earlier and much of the principles remain the same.

Several types of sausages are considered RTE and so they must meet a 5log reduction of *E.coli* O157:H7. This is done to ensure that even in the worst-case scenario of high pathogen load and resilient strains the amount of cells present will be reduced to a minimum. The smoking step exposes the meat to phenols which are antimicrobial compounds capable of causing further reductions (Alakomi et al., 2007; Hwang et al., 2009). Fermented foods are

an example of a hurdle technology where a combination of factors is used to restrict the growth of pathogens.

    iv.   Heat/Cooking

Heating or cooking processes when performed using the right temperature/time combination is considered as a lethality step (Orta-Ramirez et al., 1997). The D-value for *E.coli* O157:H7 is 0.43 min at 63°C and *Salmonella senftenberg* has a D value of 2.08 min at 63°(Orta-Ramirez et al., 1997). There is a high variation in thermal resistance between different organisms than not only depends on intrinsic properties but extrinsic properties such as substrate and type of heat (Borch and Arinder, 2002).

Several studies have been conducted using *Salmonella* spp., *Listeria* spp., and *E. coli* O157:H7 to demonstrate the effects of commercial cooking. The method of cooking has an impact as well. Double-sided grilling broiling was found to perform better than single sided grilling broiling, with a 5 log reduction when on the double sided system compared to a 1.3 log reduction of *Listeria monocytogenes* and *E.coli* O157:H7 (D'Sa et al., 2000). Murphy et al., 2002, compared frying and air convection methods of cooking. In the first 25 seconds of frying beef patties showed no change in levels of *Listeria innocua* and *Salmonella senftenberg* but there was a 7log reduction at the end of 50 seconds. When using an oven however 3.5 minutes at 288°C was needed to achieve the same kill (Murphy et al., 2002). In a study that looked at the reduction of pathogens in beef patties that were cooked by the pan grill and oven broiler method it was found that the oven broiler method was the most efficient

achieving a 5 log reduction of *Salmonella* spp., and *E.coli* O157:H7 independent of whether the patty was thawed or frozen (Manios and Skandamis, 2015). In the double-sided grill broiler heat was transmitted onto both external surfaces by conduction, in the oven broiler system heat is transmitted by conduction and convention, in the pan-grill and single sided griller broiler system heat is transferred via conduction only one side at a time and in the convection oven system heat is transferred by convection only. This shows that the method of heat transfer is important as conduction offers greater penetration of heat than convection or radiation and so conduction allows for much higher lethality rates in a reduced time period.

Strain variation is also observed between pathogens. In the study conducted by Pokharel et al., 2016, of seven STEC strains different survival rates were obtained between serogroups. Serogroups O26,O103,O111 were detected in sirloin flaps cooked at 60°C , the O157:H7 serogroup survived until 65°C (detected by PCR and on plates), the O145 serogroup was most resilient and survived a cooking temperature of 71°C (Pokharel et al., 2016). A similar study of survival of different *Salmonella* serogroups was conducted by Stopforth et al., where survival of multi-resistant drugs serogroups was higher at 60°C than non-multi drug resistant serotypes. *Salmonella* Agona was able to survive in ground beef that was cooked to a temperature of 71°C (Stopforth et al., 2017). These studies therefore highlight the importance of knowing which strain is prevalent in the food matrix and designing a cooking process sufficient to eliminate it.

Packaging

After production, all products go through packaging which serves to protect the content from the external environment. New applications applied in packaging include providing a modified atmosphere (Kudra et al., 2013; Pereira et al., 2015) or embedding active anti-microbial systems in the packaging components (Sung et al., 2013). The advances made in polymer chemistry now enable manufacture of films with varying permeability to oxygen and water vapor and can even be used to filter out light. Packaging can now be "more" tailor made to applications than ever before.

Modified Atmosphere Packaging (MAP) is obtained by modifying the composition of gases inside the product container, this can be by flushing with carbon dioxide, nitrogen or including a slow release packet. The slow release packet continually release vapors of antimicrobial compounds or gases (namely carbon dioxide) in order to maintain a modified atmosphere (Sung et al., 2013). An application of MAP was found in the extension of a traditional RTE Portuguese blood sausage-Morcela de Arroz. The sausage is generally sold without any packaging and has a shelf-life of 8 days. MAP packaged sausage however had a shelf-life of 22 days and was widely accepted by the trained consumer panel (Pereira et al., 2015). The gas combinations used in MAP also has an impact on the survival of pathogens. Barrera et al., found that ground beef when packed with 100% CO_2 there was a 0.8 log CFU/g reduction of *E.coli* O157:H7 whereas ground beef packed with 35% CO_2, 35% O_2, 30% N_2 had a decrease of 0.45 log CFU/g of *E.coli* O157:H7 (Barrera et al., 2007). It should be noted that

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the MAP system changes the microbial population present with LAB increasing in number due to the anaerobic environment (Barrera et al., 2007; Pereira et al., 2015).

Newer packaging materials are currently being developed that include antimicrobial compounds in the packaging system; the added advantage is that these not only extend shelf-life and control pathogens but can be engineered to be biodegradable (Sung et al., 2013). Plant extracts are also used as antimicrobials in packaging. In a study conducted by Sung et al.,2013, the growth rate of Listeria monocytogenes in RTE meat loaf was reduced by film that was coated with 2% garlic oil; the garlic film had a 1.98 log CFU/g load lower than the control film at the end of sixth day days when stored at 4°C (Sung et al., 2014). Other plant extracts used include rosemary oil, thyme and, oregano oil (Irkin and Esmer, 2015; Sung et al., 2013). Ravishankar et al., 2009, incorporated carvacrol and cinnamaldehyde into edible films at different levels (0-3%) and observed reductions in the population of *S.enterica* serovar Enteritidis, *E.coli* O157:H7, and *L. monocytogenes*. A level of 3% antimicrobials in film caused a 4.3-6.8 log CFU/g reduction of both *E.coli* O157:H7 and *Salmonella enterica* at 23°C whereas, a 2.3 log CFU/g reduction was seen on Listeria monocytogenes on ham stored at 23°C (Ravishankar et al., 2009). Use of antimicrobial chemicals like citrate, dissolved in gelatin and applied to paper which comes into contact with the product. Battisti et al.,2017, used citrate coated polymer at two levels; 0.5% and 1% and a reduction in the load of spoilage microbes by almost 4 log CFU/g was observed. In addition citrate coated polymers were effective at slowing down lipid oxidations (Battisti et al., 2017). Nisin when used at levels of 500µg/ml showed a 1.8-4.6 log cycle reduction of *Salmonella* Typhimurium[NAr] after 72 hours

at 4°C when applied to agar sheets used to cover chicken broilers (Natrajan and Sheldon, 2000). When used in cellulose films at 2500 IU/ml nisin was able to reduce *Listeria monocytogenes* population by 2 log CFU/g on frankfurters stored under refrigeration (Nguyen et al., 2008).

With consumers increasing demand of "more natural" components MAP and intelligent packaging systems are sure to increase in importance and occurrence. Nisin and compounds in essential plant oils have been shown to be active against multiple pathogens and so will increase in importance once more studies have been able to achieve standardized results and develop an economic way of producing such films.

Conclusion

Ground beef is a significant contributor to foodborne illness caused by beef especially when consumed undercooked. Consumer education and application of newer packaging technologies mentioned above are possible solutions. Fermented sausages if processed properly are a low risk foods and most illnesses traced to these types of food occurs due to post-processing contamination. With consumer push towards natural products interventions that use plant essential oils and naturally occurring compounds will be more widely used by the food industry. More research needs to be done on the usage of essential oil to control the growth of pathogens both as an ingredient in the product or as a component of an active packaging system. The usage of such active or intelligent packaging systems will increase in prevalence as cost goes down and efficacy of use increase.

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CHAPTER 4: PREVALENCE OF *SALMONELLA* SPP., AND *E.COLI* O157:H7 IN ORGANICALLY

RAISED CATTLE

Introduction

To ensure that consumers obtained regulated and defined products the National Organic Program (NOP) was established and the regulations governing it are covered in 7 CFR part 205. The NOP legally requires organic producers to meet certain standards in order to place the USDA Organic Seal on products. These standards address the usage of chemicals, healthcare and welfare of livestock, pest management and other practices (Coffey and Baier, 2012).

One of the beliefs that consumers hold is that organic foods are safer than conventional foods (Sofos, 2008). Harvey et al., in 2016 reported that organic foods were linked 18 foodborne outbreaks, which is low when compared to the total number of foodborne outbreaks of 7246 outbreaks of viral or bacterial related foodborne diseases from 1998-2008 (Crowe et al., 2015). Sixteen of the last eighteen total bacterial and viral outbreaks that have been associated with organic foods occurred between 2005 and 2015 (Harvey et al., 2016). Since 2005 organic sales have been also been increasing at a high rate; 29.3% increase in meat and poultry (Sustainable Food News, 2016), $4.2 billion increase in sales in 2015 (Watrous, 2016). As demonstrated by Harvey et al.,2016, the microbial safety of organic foods may be questionable especially, as increased sales is now associated with an increase in the number of outbreaks.

Escherichia coli O157:H7 and *Salmonella* species are the major pathogens associated with organic foods being responsible for 33% and 44% of all cases respectively (Harvey et al., 2016). These two foodborne pathogens are particularly damaging to the economy and are estimated to have a total cost of over $5,000 million in 2010 (Scharff, 2012). Beef cattle are reservoirs for these two pathogens and can carry them asymptomatically; making beef an excellent entry point for these pathogens into the food supply (Hsi et al., 2015). It is therefore imperative to measure the prevalence of these pathogens in beef and develop effective interventions to prevent them from causing illness.

Organic systems do not use synthetic chemical fertilizers and raw manure is frequently added to the soil in to boost its nutrient profile. Within organic production manure is derived from livestock namely; bovine, ovine or porcine and has been known to harbor *E.coli* O157:H7 and *Salmonella*. Manure has been found to be an excellent vehicle for transmission of these pathogens (Franz et al., 2005; Holley et al., 2007; Islam et al., 2004; Kudva et al., 1998). Once in the soil the survival rate of pathogens is highly varied and dependent on temperature and climate (Muirhead, 2009). Contaminated manure has been shown to effectively transfer pathogens to vegetation (Holley et al., 2007; Jacobsen and Bech, 2012).The usage of manure therefore can potentially increase the risk of contamination events especially as vegetation may serve as an oral inoculation of grazing cattle.

Livestock feed ingredients such as forage (wet and dry), grains, corn, hay and silage has been shown to be a source of both *E.coli* O157:H7 and *Salmonella* species that even allows for replication under right conditions (Dargatz et al., 2005; Krytenburg et al., 1998; Lynn et al., 1998) making it another source of pathogens in the farming environment that

may have to be monitored. Feed has also been traced to outbreaks in the past; pre-1970 *S. enterica* subtype Agona occurred rarely in the USA but in the early 1970's there was an outbreak caused by *S. enterica* subtype Agona that was traced back to contaminated fish meal that was fed to cattle (Clark et al., 1973). Davis et al.,2003, also found that strains of *Salmonella* spp., and *E.coli* O157:H7 isolated from feed could be found in environmental and fecal samples as well (Davis et al., 2003).

This study was based on an integrated livestock system that seeks to determine the prevalence of *E.coli* O157:H7 and *Salmonella* species in feed, fecal, hide and meat swabs sampled in Iowa, Minnesota, and Pennsylvania from August 2015 to March 2017.

Materials and Methods

Experimental Design and Field Operations

Research stations in Iowa and Minnesota and The Rodale Institute in Pennsylvania was used as an "on farm" environment in this study. Each location was organically verified and on a three year rotation period, small grains grazing (rye, barley) to row crop (soy beans and corn) production. The study began in August 2015 with 6-7 months old dairy beef steer. Cattle were obtained from Erlandam Farm, Greenfield (IA) for the Iowa site, Morris Research Station for the Minnesota (MN) site and the Rodale Farm for the Pennsylvania (PA) site. Herd sizes were 8, 12 and 11 for Iowa, Pennsylvania and Minnesota respectively.

Cattle were raised as per to USDA organic livestock regulations and were fed a 100% organic diet. If pasture/forage was not enough due to environmental conditions certified

organic feed was provided to the cattle. Cattle also had access to minerals and water ad libitum.

Feed and Forage Sampling

Feed and forage samples were obtained and tested for presence of *E.coli* O157:H7 and *Salmonella* species. For forage samples 100g of forage matter and feed silage that included plant foliage, roots and soil matter in order to obtain a true representation of pasture conditions. Samples were placed in sterile collection bags and shipped over night. Total mixed ratio and nutri-balancer along with mineral supplements were also sampled. Feed and pasture were sampled whenever there was a change in feeding scheme and when steers were moved onto new pastures.

Collection of Fecal Samples

Fecal samples were collected from freshly deposited cow pats on the ground. Sterile sample collection cups were provided to store and transport fecal samples. Fecal samples were shipped on ice and stored at 4°C until analysis (within 24 hours). Fecal samples were taken at the start of the study, before harvest and whenever there was a change in feed.

Hide and Meat Swabs

Hide surface samples were collected prior to harvest and meat steaks were collected after harvest using a 10x10cm template with an EZ Reach™ sponge sampler (World Bioproducts, IL, USA) and then shipped on ice. All samples were analyzed within 24 hours upon arrival. The rump was sampled for hide and the loins were swabbed on both sides for the meat samples.

Microbial Analysis

Twenty-five grams of sub-samples of feed were analyzed. Fecal samples were analyzed in sub-samples of 10g. For meat swabs 1ml of sampling buffer was squeezed out and sampled. Samples were then selectively enriched for either *E.coli* O157:H7 or *Salmonella* spp., and analyzed using miniVidas kits (bioMerieux SA, Marcy-l'Etoile, France). Samples that tested positive were then subject to confirmatory methods as prescribed by USDA and FDA BAM standards.

For *E.coli* O157:H7 samples were diluted in 1:10 ratio using Buffered Peptone Water (BPW) supplemented with Vancomycin (8mg/l) (bioMerieux SA, Marcy-l'Etoile, France), Cefixime(0.0125mg/l) (USP, MD), and Cefsulodin (10mg/l) (RPI, IL, USA) for 24±2 hours at 41.5±1°C. 0.5 ml of enriched broth was then transferred to a VIDASUP ECPT (bioMerieux SA, Marcy-l'Etoile, France) test strip and processed according to instruction provided by the manufacturer (bioMerieux SA, Marcy-l'Etoile, France). Results obtained were either positive or negative and positive results were then analyzed by further confirmatory tests.

E.coli O157:H7 colonies were identified by plating onto Sorbitol MacConkey Agar (DIFCO BD, MD, USA) with Cefixime and Tellurite (Oxiod, UK) (CT-SMAC) as colorless colonies. To isolate *E.coli* O157:H7 samples were added in a 1:10 dilution to *E.coli* broth supplemented with novobiocin and incubated at 42±1°C for 24±2 hours. A loopful of 10μl was then streaked onto CT-SMAC plates which were incubated at 42±1°C for 24±2 hours. Colorless colonies were considered as typical and 3-4 colonies per plate if present were picked at random to be

analyzed using a Dry Spot™ latex kit for *E.coli* O157 (Oxiod, UK). Agglutination with the latex reagents was considered as positive for *E.coli* O157:H7.

Salmonella detection and isolation followed a similar scheme. Samples were analyzed in a 1:10 dilution of BPW supplemented by *Salmonella* supplement and then incubated at 42±1°C for 24±2 hours. Post-incubation 1ml of enriched broth was transferred to 10ml of SX-2 broth which was incubated at 42°C for a further 24±2 hours. After the incubation period 0.5 ml of the second transfer was loaded into a VidasUP SPT kit and analysis carried out according to the manufacturer instructions. Results were obtained as either a positive or negative.

Positive results were further analyzed to confirm presence of *Salmonella* spp., using USDA. A 1:10 dilution of sample was made using mTSB with novobiocin and incubated at 42±1°C for 24±2 hours. 0.5 ml was then transferred concurrently into 10 ml of Hanja/TT broth (DIFCO BD, MD, USA) and 0.1ml into 10 ml of mRV broth. These were then incubated at 35±1°C for a 24±2 hours. Tubes were then vortexed and mixed. 10µl samples were streaked in duplicate onto Brilliant green sulfa agar (BGS) (DIFCO DB, MD, USA) and xylose lysine tergitol™ 4 agar (XLT-4) (DIFCO BD, MD, USA) and incubated at 35°C for 24±2 hours. Typical colonies were picked as per manufacture instructions. Colonies were then used to inoculate butts of Triple sugar iron (TSI) and lysine-iron agar (LIA) which were then incubated with loosened caps at 35±2°C for 24 hours. The LIA and TSI stabs were examined as a set for positives in accordance with guidelines set by the manufacturer.

Results

Feed Samples

A total of fifty three feed samples from all locations was tested for the presence of *E.coli* O157:H7 and *Salmonella* spp. *E.coli* O157:H7 and *Salmonella* spp., were not detected in feed samples from Iowa throughout the study. Overall five samples tested positive for *E.coli* O157:H7 and one for *Salmonella* spp., as can been seen in Table A1. Geographically the results differed numerically in that no samples from Iowa tested positive for either pathogen. Three feed samples out of sixteen tested positive for *E.coli* O157:H7 from Minnesota (Table A2) and these were obtained from pasture in June and July 2016. They were taken two months prior to harvest of the cattle. Two out of twenty feed samples from Pennsylvania tested positive *E.coli* O157:H7 (Table 3) in August during the first round of feed sampling and these were total mixed ration. The sole positive sample for *Salmonella* spp., in feed was from Pennsylvania (Table A3) which was obtained in August during the first round of feed sampling and was also total mixed ration. All positive samples were isolated in the warmer months of June, July, and August.

Fecal Samples

A hundred and twenty four fecal samples were analyzed throughout the study. Samples were taken post weaning, whenever there was a change of feed and prior to harvest. Nine samples tested positive for *E.coli* O157:H7 and four tested positive for *Salmonella* spp., (Table A4)*.* All the positive samples for *Salmonella* spp., were from Iowa in the month of August (Table A5) and were taken prior to harvest. The *E.coli* O157:H7 were isolated from

one sample from Minnesota (Table A6) which was obtained during the first round of sampling in August in the post-weaning stage. Eight samples Pennsylvania tested positive for *E.coli* O157:H7(Table 7). The Pennsylvania samples that tested positive were taken towards the end of the study prior to harvest of the cattle. All Iowa fecal samples tested negative for *E.coli* O157:H7 (Table A4).

Hide Samples

Sampling of hide occurred prior to harvest only. Hide samples (N= 43) were obtained in July and August 2016 from Minnesota and Iowa (August 2016 only). There were no hide samples from Pennsylvania due to harvest schedule. *E.coli* O157:H7 was not detected on any hide sample whereas *Salmonella* spp., was detected on eight samples which were all obtained in August (Table A8). Four of the positive samples were from Minnesota and the rest were from Iowa (Table A9 & A10).

Meat Samples

E.coli O157:H7 or *Salmonella* spp., was not detected in any meat samples obtained (Table A11).

Discussion

Feed samples

Positive feed samples for both pathogens were obtained in the warmer months of June, July and August in Pennsylvania and Minnesota which are the months frequently associated with higher prevalence rates (Barkocy-Gallagher et al., 2003; Hussein, 2007; Lahti

et al., 2003; Williams et al., 2015) across several countries like United States of America, Finland and Australia. *Salmonella* spp., was detected in 1.89% of the 53 samples which is much lower than expected when compared to Krytenburg et al.,1998, who detected *Salmonella* spp., in 9.8% of feed samples that included several types of feed components; obtained between July and November from dairy farms and feedlots (Krytenburg et al., 1998). In a more recent study by Davis et al.,2003, *Salmonella enterica* was isolated in 0.8% of feed components that included apple waste, fish meal, alfalfa hay, grain mill waste, crushed corn, hay, cotton seed, rye pellets, corn silage, alfalfa silage, beet pulp, pellets and pea flour (Davis et al., 2003) and is much closer to the value obtained in this study. The lower prevalence could be due to the fact that Krytenburg et al.,1998, sampled a wide variety of feeds and in much greater numbers than the current study; 295 feed samples across 10 types of feed components versus the current study of 53 feed samples. Some of the feeds studied by Krytenburg et al.,1998, were alfalfa, alfalfa hay, chopped grass hay, hay, maize and grass silage, rye pellets, oat pellets, whey, rolled barley, rolled/ cracked/ ground maize, canola and cotton seed to name a few. *E.coli* O157:H7 was found in three samples from Minnesota that were obtained on pasture and 2 samples in Pennsylvania from total mixed ratio. Overall *E.coli* O157:H7 was detected in 9.43% of the samples (pasture and total mixed ratio were positive) and is much higher than found in studies conducted by Davis et al., 2003, who obtained 0.4% from feed mill samples (Davis et al., 2003) and 0% from feed samples obtained from farms feed mill, and dairies in a study conducted by Lynn et al., in 1998. The prevalence observed in this study is closer to the 14.9% prevalence obtained by Sargeant et al.,2004, although they examined feed from feed bins in feedlots (Sargeant et al., 2004).The lower prevalence in the

studies by Davis et al.,2003, and Lynn et al.,1998, could also be due to them examining feed components before being offered to cattle rather than feed in bins. The *Salmonella* prevalence of 1.89% found in feeds was similar to the study conducted by Davis et al.,2003, on feed samples obtained from conventional dairy farms in Washington and North-West Idaho. *E.coli* O157:H7 prevalence was 9.43% and was closer to the prevalence obtained on feedlots by Sargeant et al., 2004.

Fecal samples

A total of hundred and twenty-four fecal samples were examined and *E.coli* O157:H7 was isolated from nine samples; eight from Pennsylvania in January and one from Minnesota in August. *E.coli* O157:H7 was isolated in 7.26% of samples. A study by Reinstein et al., 2009, on organically raised cattle, observed a large variation of prevalence rates across sampling days with a minimum prevalence rate of 0 to a maximum rate of 24.4%, with an average rate of 9.3% (Reinstein et al., 2009). A similar study conducted on grass fed cattle in Australia observed a prevalence rate of 10% (N. Fegan et al., 2004). These studies however used molecular techniques such as PCR and Immunomagnetic bead separation for enrichment which have been shown to be more sensitive (Eriksson et al., 2007). Jacob et al.,2009, however found a lower prevalence rate (5.1%) of *E.coli* O157:H7 in an experimental setting of cows fed dried distillers grains or dry-rolled corn. The total number of cows examined was twenty eight, making it comparable in size to the current study (Jacob et al., 2009). Cho et al., 2006, examined cows in Minnesota organic dairy where a prevalence rate of *E.coli* O157:H7 was found to be 7.4% (Cho et al., 2006). The prevalence rate of 7.26% is therefore is not out

of bounds for this region. *Salmonella* spp., was isolated only from four Iowa samples in August. The overall prevalence rate of *Salmonella* spp*.,* of 3.26% was numerically lower than in study conducted by Fossler et al., 2005, who detected *Salmonella* spp., in 5.2% of fecal samples collected from organic dairy farms in Minnesota and Wisconsin (Fossler et al., 2005). A study conducted on feedlot cattle in Nebraska where prevalence was calculated to be 5.4% (Schmidt et al., 2015). A study of grass-fed cattle in Australia by Fegan et al., 2004, obtained a prevalence rate of 4.5% of *Salmonella* in beef cattle feces (Narelle Fegan et al., 2004) and this is closer in value to the 3.26% obtained in this study. The prevalence rate obtained for *Salmonella* spp., in fecal samples is not close to values obtained in prior studies and, the lower rate observed could be due to the number of cattle involved as herd size was found to be a significant factor in farms with higher prevalence rates (Fossler et al., 2005; Habing et al., 2012). Numerically the prevalence rates are not far apart and could be in fact be a result of differences in herd size, number of samples and techniques employed. The prevalence of *E.coli* O157:H7 in feces is similar to values obtained for grass-fed or feedlot cattle and agrees with the study conducted by Cho et al., in an organic dairy in Minnesota. *Salmonella* prevalence was numerically lower than previous studies conducted in the region on organic and conventional production systems.

Hide samples

Forty-three hide samples from cattle in Iowa and Minnesota were sampled before harvest. No samples tested positive for *E.coli* O157:H7 whereas four samples each from Iowa and Minnesota tested positive for the presence of *Salmonella* spp. The overall prevalence

rate for *Salmonella* spp., on hides was 18.6%. Most studies isolate *E.coli* O157:H7 at higher rates (30-60%) from hide during harvest (Arthur et al., 2007; Barkocy-Gallagher et al., 2003; Brichta-Harhay et al., 2008). Barkocy-Gallagher et al., 2003, examined *E.coli* O157:H7 on hides in a commercial beef processing plant that sourced cattle from the Midwest and observed positive samples in 60.6% of hides whereas Brichta-Harhay et al.,2008, examined hides of cattle from processing facilities across the USA and obtained 33.3% of positive hide samples. The current results however, are in agreement with a study conducted by Barham et al., 2002, on feedlot cattle in Texas, detected a decrease in *E.coli* O157:H7 in hide samples after transport and, just before harvest (Barham et al., 2002). *Salmonella* spp., prevalence was much higher in hide samples (18.6%) taken before harvest than fecal samples (3.33%), a pattern observed by both Barham et al.,2002, and Barkocy-Gallagher et al.,2003, who saw an increase of 89 % (hide) versus 46% (fecal) (Barham et al., 2002) and 71% (hide) versus 4.4% (fecal). The prevalence rate on hides observed is much lower in this study when compared to other studies which have rates from 6% to 93.8% (Arthur et al., 2007; Barham et al., 2002; Barkocy-Gallagher et al., 2003; Brichta-Harhay et al., 2008) and are highly variable. An Australian study on *Salmonella* prevalence found similar results with 92% hide and 7% fecal samples obtained from organically raised cows being positive for *Salmonella* spp., (Fegan et al., 2005)Variation is seen in season (Brichta-Harhay et al., 2008) and before/after transport (Barham et al., 2002). The *Salmonella* spp., prevalence rate although low in comparison to most studies in organic and conventionally raised cattle fit the trend of being higher in hide than fecal samples.

Meat samples

All meat swabs were negative for either pathogen. This is could be due to the relatively low occurrence of pathogens during the production period. The swabs were taken post-chilling which, considerably reduces the prevalence of both pathogens (Barkocy-Gallagher et al., 2003; Brichta-Harhay et al., 2008; Hauge et al., 2015). Barkocy-Gallagher et al., 2003, observed very low rates of occurrence of both *E.coli* O157:H7 and *Salmonella* spp., in carcasses of Midwestern cattle post chilling; 1.2% and 0.1% respectively. Brichta-Harhay et al., 2008, saw a similar drastic reduction in *Salmonella* prevalence post chilling – 0.8% despite having much higher prevalence rates on hide and fecal samples. Similar to the 0% prevalence in this study Fegan et al.,2005, obtained a 0% prevalence of *Salmonella* post chilling in organically raised Australian cattle (Fegan et al., 2005). Hauge et al.,2015, saw almost a tenfold reduction (11% to 1%) post chilling in total *E.coli* populations in their study of two Norwegian abattoirs though there was no indication of whether the cattle were organically or conventionally raised. The rare incidence of pathogens in the cattle from earlier sampling points and the interventions applied could have led to a decrease in the low number of pathogens already present, which is why the meat swabs were observed to be negative for both pathogens.

Conclusion

The prevalence rate for *E.coli* 157:H7 for feed was 9.43% which was higher compared to previously studies performed on feed taken from feed mills but comparable to the prevalence level found in feedbins of feedlots. *Salmonella* spp., prevalence was lower than

previous studies at 1.89% for feed but was numerically close to values obtained from feed samples taken from dairy farms in Washington and North-West Idaho. Isolation of *E.coli* O157:H7 from feces was similar to a study performed on organic dairy cows in Minnesota with 7.26% of the samples in this study testing positive. Fecal prevalence rate of 3.26% of *Salmonella* spp., was much lower than previous studies conducted on different production systems and can probably be attributed to the size of the herd which was much smaller than other studies (less than 50 head). No *E.coli* O157:H7 was detected in hide samples taken prior to harvest whereas previous studies indicated a higher prevalence in hide. *Salmonella* prevalence on hide was the highest with 18.6% testing positive and this trend matched older studies conducted in abattoirs. *Salmonella* was not isolated from any meat samples which was observed in organic cows of an Australian study with a sample size. The drastic reduction of both pathogens post the chilling period as seen literature and the low prevalence's obtained in this study are likely the reason as to why no pathogen was detected in meat samples.

Overall the study detected much lower prevalence rates of *E.coli* O157:H7 in feed and fecal samples but they were not numerically very different from studies conducted on organic and conventional systems in the USA and presence in feed was comparable to presence in feedlots. The absence of *E.coli* O157:H7 on hide samples was noticeably different but could be explained by the reduction in population of *E.coli* O157:H7 seen after transport. *Salmonella* prevalence on hides was higher than feed or fecal and this is seen in many studies in both conventional and organic systems. Meat swabs all tested negative and this was

attributed to the fact that most studies recorded a severe decrease in pathogen levels at the

post-chilling stage.

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CHAPTER 5: SUMMARY AND CONCLUSION

The organic market is seeing increasing demand as evidenced by the high growth rate; organic sales from farms rose by 72% in 2014 from 2008, in 2015 organic sales was at 43.3 billion which is a growth of 11% from 2014. Consumers associate organic foods with higher safety, better health and a nutrition profile as found out by a survey conducted by ACNielson in 2005. While scientific reports find minimal to no difference, it is wise to remember that the Food Industry is consumer driven and consumer demands must be met. Consumer choices are complex and several factors must be taken into account like; price, ethical background, health consciousness, etc., to name a few. Organic Livestock Production is more animal welfare oriented and sustainable which are key drivers for adoption by consumers. Organic methods in fact, have been shown to be more sustainable than conventional methods with regards to environmental impact. These factors work favorably in the minds of consumers and therefore organic products are more likely to stay and increase in market share with time.

As stated earlier organic foods are associated to be more safe than conventional products by consumers but this is not the case as foodborne illnesses have been traced to organic foods as well. Most of the outbreaks occurred post 2000 which goes in line with the increasing sales trend. Organic foods and organic production systems must therefore be studied and analyzed for potential food safety risks in order to produce wholesome goods and ensure consumer safety.

The study conducted on an Integrated Livestock System is an Organic method of Livestock Production that examined feed, fecal matter, hide and meat of organically managed dairy steers for the presence of *E.coli* O157:H7 and *Salmonella* spp., in Iowa, Minnesota and

Pennsylvania from August 2015 to March 2017. The prevalence rates observed were numerically lower than other studies performed in the region for both pathogens. No pathogens were detected in the meat samples which shows that the product was safe for consumption by consumers. Nutritional aspects of the meat were not examined in this study and could be possibly looked at in the future. The study however found no major difference in prevalence rates of pathogen occurrence when compared to conventional systems. The study was conducted using fewer cattle (< 100) but results were comparable to larger studies. More research should be done to ensure the safety of the complex system that is Integrated Livestock Production.

Amongst beef products ground beef is notorious for its involvement in outbreaks of foodborne illnesses especially *E.coli* O157:H7 which is colloquially known as "Hamburger Disease". Beef trim used should be monitored to ensure no pathogens are present. Consumers should be advised and educated on consumption and preparation of ground beef to reduce risk of foodborne outbreaks. Fermented sausages have a low food safety risk and this is attributed to the multiple hurdles associated with production of sausage such a temperature, exposure to phenolic compounds, presence of Lactic Acid Bacteria and antimicrobial compounds in spices used for flavoring. The major bacterial pathogens associated with Fermented Sausages are *Listeria monocytogenes*, *Staphylococcus aureus* which are indicative of post-processing contamination. As long as the degree hour requirements are met and post-processing contamination is avoided the product should be free from pathogens. New technology associated with packaging techniques such as Modified

Atmosphere and Smart packing with antimicrobial activity are also employed and being developed to ensure safety and extend shelf-life of the product.

APPENDIX: TABLES IN CHAPTER 4

Table A1. Presence of *E.coli* O157:H7 and *Salmonella* spp., in Feed by Month in All Locations

Pathogen	*E.coli* O157:H7		*Salmonella* spp.	
Month	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Aug-15	2 (13.33)	13 (86.67)	1 (6.67)	14 (93.33)
Nov-15	0	18	0	18
May-16	0	2	0	2
Jun-16	2 (33.33)	4 (66.67)	0	6
Jul-16	1 (33.33)	2 (66.67)	0	3
Sep-16	0	7	0	7
Jan-17	0	2	0	2
Total	5 (9.43)	48 (9.57)	1 (1.89)	52 (98.11)

Table A2. Presence of *E.coli* O157:H7 and *Salmonella* spp., in Feed by Month in Minnesota

Pathogen	*E.coli* O157:H7		*Salmonella* spp.	
Month	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Aug-15	0	3	0	3
Nov-15	0	4	0	4
Jun-16	2 (33.33)	4 (66.67)	0	6
Jul-16	1 (33.33)	2 (66.67)	0	3

Table A3. Presence of *E.coli* O157:H7 and *Salmonella* spp., in Feed by Month in Pennsylvania

Pathogen	*E.coli* O157:H7		*Salmonella* spp.	
Month	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Aug-15	2 (25)	6 (75)	1 (12.5)	7 (87.5)
Nov-15	0	8	0	8
May-16	0	2	0	2
Jan-16	0	2	0	2

Table A4. Presence of *E.coli* O157:H7 and *Salmonella* spp., in Feces by Month across all Locations

Pathogen	*E.coli* O157:H7		*Salmonella* spp.	
Month	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Aug-15	1 (4.35)	22 (95.65)	0	23
Nov-15	0	27	0	27
May-16	0	10	0	10
Jul-16	0	14	0	14
Aug-16	0	14	4 (28.57)	10 (71.43)
Sep-16	0	7	0	7
Jan-17	8 (42.11)	11 (57.89)	0	19
Feb-17	0	4	0	4
Mar-17	0	6	0	6
Total	9 (7.26)	115 (92.74)	4 (3.26)	120 (96.77)

Table A5. Presence of *E.coli* O157:H7 and *Salmonella* spp., in Feces by Month in Iowa

Pathogen	*E.coli* O157:H7		*Salmonella* spp.	
Month	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Aug-15	0	4	0	4
Nov-15	0	8	0	8
Aug-16	0	14	4 (28.57)	10 (71.43)
Sep-16	0	7	0	7
Jan-17	0	4	0	4

Table A6. Presence of *E.coli* O157:H7 and *Salmonella* spp., in Feces by Month in Minnesota

Pathogen	*E.coli* O157:H7		*Salmonella* spp.	
Month	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Aug-15	1 (10)	9 (90)	0	10
Nov-15	0	8	0	8
Jul-16	0	14	0	14

Table A7. Presence of *E.coli* O157:H7 and *Salmonella* spp., in Feces by Month in Pennsylvania

Pathogen	*E.coli* O157:H7		*Salmonella* spp.	
Month	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Aug-15	0	9	0	9
Nov-15	0	11	0	11
May-16	0	10	0	10
Jan-17	8 (53.33)	7 (46.67)	0	15
Feb-17	0	4	0	4
Mar-17	0	6	0	6

Table A8. Presence of *E.coli* O157:H7 and *Salmonella* spp., in Hide by Month all locations

Pathogen	*E.coli* O157:H7		*Salmonella* spp.	
Month	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Jul-16	0	14	0	14
Aug-16	0	29	8 (27.59)	21 (72.41)
Total	0	43	8 (18.60)	35 (81.40)

Table A9. Presence of *E.coli* O157:H7 and *Salmonella* spp., in Hide by Month in Iowa

Pathogen	*E.coli* O157:H7		*Salmonella* spp.	
Month	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Aug-16	0	15	4 (26.67)	11 (73.33)

Table A10. Presence of *E.coli* O157:H7 and *Salmonella* spp., in Hide by Month in Minnesota

Pathogen	*E.coli* O157:H7		*Salmonella* spp.	
Month	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Jul-16	0	14	0	14
Aug-16	0	14	4	10

Table A11. Prevalence of pathogens in Feed, Fecal, Hide and Meat Samples

Pathogen	*E.coli* O157:H7			*Salmonella* spp.		
Sample Type	Positive	Negative	Prevalence (%)	Positive	Negative	Prevalence
Feed	5	48	9.43	1	52	1.92
Fecal	9	113	7.38	4	118	3.28
Hide	0	43	0	8	35	18.6
Meat	0	28	0	0	28	0