PRESENCE OF *SALMONELLA*, *ESCHERICHIA COLI* O157 AND

CAMPYLOBACTER IN SMALL-RUMINANTS

By

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Table of Contents

Abstract

Meat derived from goats and lambs is an important and often underappreciated protein source in the U.S., but it is especially important in developing countries challenged with food insecurity. While extensive studies have been conducted examining pathogen prevalence in beef, pork and poultry species, less research is available about pathogen presence in small-ruminants. In developing countries, small-ruminants often co-exist with humans in living and work conditions, making cross contamination and frequent contact with fecal matter a common occurrence. In the U.S., the popularity of small ruminants as a protein source is growing. Additionally, many small-ruminants are considered pets presenting potential pathogen exposure to family members.

Salmonella, Campylobacter and *Escherichia coli* O157 are all pathogens of concern known to be found in livestock. Understanding the presence of these pathogens in small-ruminants can influence processing method improvements for these species to minimize food safety risks.

This study had three main objectives: 1) To determine pathogen presence in small-ruminants on hide and fecal samples collected from U.S. and international sources; 2) *Salmonella* presence in small-ruminant carcass surface and retail samples from the U.S. and international sources; and 3) *Salmonella* presence found in lymph nodes from sheep and goats. Samples were collected at abattoirs and farms located in California, New Mexico, Texas, the Bahamas and Mexico over a 14 month period. Samples were processed using a combination of traditional culturing methods and real-time PCR. All presumptive positive samples were confirmed by isolation and biochemical analysis.

Campylobacter, E. coli O157 and *Salmonella* were detected in samples collected from both sheep and goats. *Salmonella* was detected in 17.11% of hide samples, 13.91% of fecal samples, 16.82% of retail samples, and 1.94% - 9.62% of lymph nodes and up to 5.20% on carcass surfaces. *E.* coli O157 was present in 1.50% of hide samples and 15.30% of fecal samples. *Campylobacter* was found in 80.68% of fecal samples. The results indicate the presence of potential pathogens from small-ruminant sources. Further study of the trends of these pathogens through season, geographical location and management conditions, as well as control measures, are important to better understand the risks associated with these pathogens present in small-ruminants.

List of Tables

Chapter 1: Introduction

According to the 2011 CDC estimates, illness acquired from food affect one in six Americans each year, ultimately causing 128,000 hospitalizations and 3,000 deaths annually (CDC, 2014b). Nontyphoidal *Salmonella* and *Campylobacter* are both ranked within the top five pathogens responsible for foodborne deaths, hospitalizations and illnesses (CDC, 2014b). Together, *Campylobacter* and *Salmonella* are the two most commonly isolated bacterial pathogens from a human patient with diarrheal symptoms (Acheson & Hohmann, 2001; CDC, 2011).

Livestock can be either considered direct or indirect contributors to foodborne illness. Indirect contribution comes from contamination of water used for irrigation, cross-contamination between products or the passing of the pathogen through an animal shedding the organism. Indirect contamination sources can easily be water troughs, feed sources, pests, wildlife, animal housing, transportation equipment or processing equipment (Doyle & Erickson, 2006). Microorganisms can directly contaminate food sources through their presence in ground meat, processed foods or milk. Direct contamination can also occur when a human touches or comes into contact with the animal. Small-ruminants are popular pets in the US and are commonly found in petting zoos, which presents an opportunity for direct contact.

Research has shown *Salmonella* can be harbored in lymph nodes of cattle, with the potential to be incorporated and therefore contaminate ground trim intended for consumption (Arthur et al., 2008). Cattle have been attributed as the most important reservoir of *Escherichia coli* O157:H7 and therefore, an increased threat for human

infection from foodborne sources (Pires et al., 2009). An estimated 80.3 million global cases of foodborne human illness each year are credited to *Salmonella* infection originally from livestock sources (Majowicz et al., 2010).

The three major foodborne bacterial targets that have had, and continue to have, the spotlight for research efforts, surveillance and discussion in the food industry relative to products derived from livestock are *Salmonella* spp., *Campylobacter* spp. and shiga-toxin producing *E. coli* (Newell et al., 2010). These three organisms and their targeted research, however, have focused mostly on cattle or pigs, with less application in small-ruminants. There is limited available research about the presence and impact of *Salmonella* contamination on goat meat and goat carcasses (Duffy et al., 2009). Similarly, while some data exists about the prevalence of *Campylobacter* in many other livestock species, there is very limited published research about the prevalence of this organism in sheep and goats, especially examining the risk of transmission to humans (Horrocks et al., 2009).

Sheep and goats are often linked together when studying microbial profiles in developing countries because they are both small-ruminants associated with cultural and niche markets. In developing countries, small-ruminants and humans often live in close quarters with shared resources, potentially unhygienic processing facilities, and frequent exposure to any pathogens being shed (Mpalang et al., 2014). Understanding the prevalence of pathogens specifically involved with the production and harvesting of small-ruminants allows processors to more effectively focus their efforts on control interventions for safe food production.

This project had three objectives, with an overall goal to determine the presence of pathogens found in live sheep and goats and their meat products from the Bahamas, Mexico and United States. Objective one evaluated pathogen presence in hide samples from sheep and goats as well as the presence of *Salmonella, E. coli* O157 and *Campylobacter* found in small-ruminant fecal samples. The second objective was to evaluate retail small-ruminant samples and carcass surfaces for *Salmonella*. Finally, the third part of this study evaluated three different lymph nodes from small ruminants for *Salmonella* presence in sheep and goats.

Chapter 2: Review of literature

Small-Ruminants as a source of meat

History and background

Goats were the first farm animal to be domesticated, somewhere between 8000

B.C. and 7000 B.C. (Devendra & Solaiman, 2010). Since their domestication, goats have

played an important role in the nutrition, economy, religion and traditions of various

cultures across the world (Boyazoglu et al., 2005). Today, goats are a versatile specie

beyond their use as a protein source. Goats are frequently used for browsing, while their

milk, fiber and skins have value in a variety of markets (Devendra & Solaiman, 2010).

Historically sheep are well known as a meat and fiber source, but they also have

established roles in milk production, pharmaceutical production and browsing. Browsing

refers to the biological control of vegetation, bushes, trees and shrubs by allowing goats

or sheep to consume the brush (Abaye, 2011).

Based on total animal numbers, in 1996 sheep and goats represented the second

and fourth largest livestock species globally, respectively, with the majority of the goats

located in developing countries (Morand-Fehr & Boyazoglu, 1999). In 2007, the total

goat population worldwide was approximately 851 million, composed of 1,156 different

breeds (Devendra, 2010). This includes goats purposed for meat, as well as dairy, fiber,

fancy and multi-purpose indigenous goats. The FAO estimate of sheep and goats existing

worldwide was just over 2 billion in 2012, with a growth of 20 million head between

1990 and 2012, compared to 1.6 billion cattle and buffalo (FAO, 2014a). In 2013, meat

derived from sheep and goats accounted for approximately 13.9 million tons of recorded

meat consumption, an increase of 0.5% from the previous year (FAO, 2014b).

As of 2011, the world goat meat production was 5.2 million tons which equates to

430 million goats slaughtered (FAO, 2011). Compared to the 110 million tons of pork, 62

million tons of beef and 89 million tons of chicken, goat represents a relatively small

percentage of the quantity of total meat consumed (FAO, 2011). But goat consumption is

widespread across countries where pork, beef and chicken are consumed at lower

quantities. In 2010, approximately 90% of the total population of goats in the world

resided in developing countries (Lu et al., 2010). According to the summary of the

International Conference on Goats in 2010, the population growth rate of humans in the

50 least developed countries of the world is exceeded by the goat population growth rate

in those same countries (Devendra, 2010). As of 2013, 54% of all recorded sheep and

goat meat was produced in Asia, 19.7% in Africa, 12.3% in Europe and 4.5% in the

Americas (FAO, 2015). A study in Mexico found that the goat inventory in Mexico

decreased by 9.1% from 1994 to 2004, but the country's production of goat meat

carcasses increased by 9.0% over that period (Rebollar-Rebollar et al., 2006).

With limited commercialized goat meat production in most developing countries,

the majority of production is done on a small scale through local abattoirs, butcher shops

and small meat markets. In 2006, goat farms in Brazil averaged only 26 goats per farm

(Madruga, 2011). In Mexico, goat production usually consists of more traditional

management methods which include small scale and diverse farming operations, or

browsing in areas that are not easily grazed by other species (Rebollar-Rebollar et al.,

2006). In Africa goat keepers generally manage small herds on farms with a variety of other species (Peacock, 2005).

One of the reasons goats are such a successful species in developing countries is because goats have a relatively short gestation period (5 months), they are easier to feed and maintain than larger livestock, and are able to thrive in areas with more limited feed availability, when compared to the needs of other species. These characteristics are especially valuable in poor areas where drought and difficult weather conditions prevail (Peacock, 2005).

The unstructured management conditions of most goats in these countries limits the accuracy of estimating actual goat production statistics. In Africa, the informal, untaxed and unstructured marketing and sale of goat meat and goat products impedes the accuracy of calculating how goats contribute to the national economy, making their value largely underestimated (Peacock, 2005). Another limit to the accuracy of goat production statistics, and limit to the widespread acceptance of goat, is the negative stigmatism associated with goats in some cultures. Many African cultures continue to associate cattle with wealth, and goats with poverty (Peacock, 2005). This negative connotation can deter countries from making policies to regulate or monitor goat meat production. Additionally, it deters more affluent communities from exploring goat meat as a viable protein source, even from within their own country.

South African Boer goats were imported into the U.S. in 1993 through cooperation with several organizations in the U.S. and Canada. As an efficient and high yielding meat breed, their arrival in the U.S. made goat meat a more available and viable

option (Oman et al., 2000). At the same time government subsidies for fiber were

discontinued, so numbers of angora and mohair goats in the U.S. declined, while the

ethnic diversity of Americans increased, which helped contribute to the growth of meat

type breeds within the goat industry (Sahlu et al., 2009). Following the discontinuation of

the National Wool Act, incentives for wool production also decreased, consequently

increasing the popularity of hair sheep with U.S. producers (Williams et al., 2008).

Over the 20 years that Boer goats have been in the U.S. their population has

exceeded the number of Boers in South Africa (Sahlu et al., 2009). The presence of more

meat type goats in the U.S. enables better production of goat meat. With the population

increase of ethnic groups in the U.S. and their increased power in the marketplace, the

demand for goat access and available products has grown (McMillin & Brock, 2005).

Goat products internationally

Goat meat or chevon prepared at different ages and in different cultures is known

by slightly different names (Madruga, 2011). "Cabrito" is goat eight to 12 weeks old,

traditionally consumed by Mediterranean countries, Latin America and Western Europe.

African, Middle Eastern and Southwest Asian countries generally consume young goats

(12-24 months old). Africa and India also have a developed market for older goats,

between two and six years of age. Very young slaughtered goats in South Africa are

sometimes referred to as cabretto (Casey & Webb, 2010). In Latin America, "buchada

caprina," is a goat by-product that uses heart, lungs, liver, intestines, blood, rumen and

kidneys (de Queiroz et al., 2013). The Banjara tribe in India, collects goat or lamb blood

for raw consumption, sometimes mixed with bile (Kumar et al., 1973). In the U.S. goat

meat is generally sold as imported frozen meat, whole carcass or cubed bone-in cuts

(McMillin & Brock, 2005). Current total market sheep and lamb inventory in the U.S. is

approximately 1.35 million head, with market goat and kid inventory estimated at

471,000 head (NASS, 2015).

Factors influencing goat and lamb meat consumption

Developed countries, especially those in Europe, have increased their overall

acceptance and consumption of goat milk and meat in the last ten years because of the

nutritional and ecological value of goats (Boyazoglu et al., 2005). Within the U.S., goat

meat consumption is repressed because of unfamiliarity and the fact that many Americans

are unaccostumed to and therefore, not keen on the flavor profile of goat meat. A survey

of consumers fed goat and lamb in the U.S. found that foreign consumers were overall

more likely to rank goat and lamb samples higher on the palatability scale than domestic

consumers (Griffin et al., 1991). One of the most limiting factors of goat milk and meat

consumption is the distinct taste of goat products (Boyazoglu et al., 2005). Changes in

amino acids during the cooking process is one studied theory about the unique flavor

profile that can be found in goat meat (Madruga et al., 2010). Studies have shown that

young goats and lambs are more palatable than older animals (Batcher et al., 1969; Smith

et al., 1978). Some sensory work has been done to show flavor and aroma differences in

various breeds of sheep and goats (Tshabalala et al., 2003).

The limits of seasonal availability, with the current status of goat production in the U.S. affects the value and ultimately cost of the product (McMillin & Brock, 2005). This is especially true around ethnic holidays that create an increased demand for goat as part of traditional meals. In the U.S., there is opportunity to expand the goat industry in future years because goats are well aligned with the increasing consumer demand for natural, healthy and ecologically positive food options (McMillin & Brock, 2005).

Domestic and global production of small-ruminants

In 2013, an estimated 13.9 million tons of lamb were produced globally, a slight increase from lamb production in 2012 (FAO, 2014b). While compared to beef, pork or poultry consumption this number may seem insignificant, however, small-ruminants are still an important and valued protein source. Since 1990, China has been the leading producer of combined goat and lamb for meat consumption, followed by India, Australia and New Zealand (FAO, 2015). Under combined federal and state regulations, 779,000 goats were inspected in the U.S. in 2010 (FSIS, 2013). Globally, per capita consumption of lamb, goat and mutton was 4.17 pounds in 2007, an increase from 3.95 in 1965 (Brester, 2012). The U.S. annual consumption is less than a pound per person, compared with 5.5 pounds per capita in Africa, and 26 pounds in Australia (Brester, 2012). In the U.S. older lambs or low quality cuts often are directed to pet food markets (Jones, 2012). The increasing focus and attention on food safety within the pet-food industry makes it important to know the microbial prevalence on ingredients currently sourced for pet food (Taylor, 2012).

Processing methods

Harvest of goats varies based on age, size and cultural traditions. In Australia, for example, many facilities process goats with 'skin-on' by using a scald, tumble and singing method similar to traditional hog processing methods (Duffy et al., 2009). Scalding or singeing of the goat carcass is also preferred by some Asian, Caribbean and African cultures (Stanton, 2012). Some sheep research has been done to see if shearing or washing the fleece of lambs prior to processing could decrease the microbial load entering the abattoir. In Norway, unshorn lamb carcasses had a significantly higher frequency of *Escherichia coli* found then carcasses of shorn lambs (Hauge et al., 2011). Sheep and goats are sometimes processed with an inverted dressing system, which has been shown to have a lower prevalence of contamination when compared to the conventional system (Bell & Hathaway, 2008). Globally many of the sheep and lambs are processed in small non-commercialized systems, therefore, a variety of methods is likely employed, based on available resources.

Salmonella

Overview of *Salmonella*

Salmonella is a gram-negative, facultative anaerobe, bacteria belonging to the *Enterobacteriaceae* family (D'Aoust & Maurer, 2007). Classification of *Salmonella* organisms is done based on structural components of the organisms, specifically the lipopolysaccharide (O), flagellar protein (H) and the (Vi) capsular antigens if they are present (Iowa State University, 2005). As a genus, *Salmonella* is comprised of two

species, *S. enterica* and *S. bongori,* with *S. enterica* further divided into six subspecies, traditionally numbered with roman numerals: *S. enterica* subspecie *enterica* (I), *S. enterica* subspecie *salamae* (II)*, S. enterica* subspecie *arizonae* (IIIa)*, S. enterica* subspecie *diarizonae* (IIIb)*, S. enterica* subspecie *houtenae* (IV) and *S. enterica* subspecie *indica* (VI) (Popoff et al., 2000). These subspecies can be further divided into over 2,500 serotypes (CDC, 2011). *S. enterica* subspecies *enterica* account for almost 60% of *Salmonella* serotypes identified (Popoff & Le Minor., 1997).

As of 2007, the five most reported serotypes isolated from humans in the U.S. were *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Heidelberg, *Salmonella* Newport and *Salmonella* Hadar, which together accounted for 66% of *Salmonella* isolates reported to the CDC (Olsen et al., 2001). *Salmonella* Enteritidis and *Salmonella* Typhimurium continue to be the two most common serotypes that cause foodborne illness in the U.S. (FSIS, 2015).

Epidemiologically *Salmonella* spp. are frequently categorized in three groups: those that infect humans only, serovars which have adapted to specific hosts (for example, certain serovars are known to be contracted from only poultry, cattle, equines, porcines, etc.) and serovars which have no specific host preference making them easily shared between hosts and species (Jay et al., 2005). Some examples of host specific *Salmonella* species can be seen in Table 1.

Aside from its complex nomenclature, *Salmonella* spp. are also known for their ability to adapt and survive in a multitude of diverse mediums. *Salmonella* spp. can be motile or nonmotile, optimally growing at 37°C, and shown to be able to use a wide

range of organic substrates for energy by either respiratory or fermentative pathways (Montville et al., 2012). While the optimum growth pH for this organism is 6.5-7.5, *Salmonella* spp. have successfully been shown to be viable at pHs ranging anywhere from 4.5 to 9.5 (Montville et al., 2012).

Similar to many other pathogens, immunocompromised individuals such as children or the elderly are more at risk for succumbing to an infection from *Salmonella* than healthy average adults. The infectious dose for *Salmonella* spp. can vary depending on the serovar, anywhere from <10 organisms of a highly virulent serovar or sometimes as high as 100,000 of a less virulent strain before causing infection (Kapperud et al., 1990; McCullough & Elsele, 1951).

Food safety relevance

Within the U.S. *Salmonella* spp. are estimated to affect 1.0 million people, ultimately causing 11% of total foodborne illness, 30% of foodborne hospitalizations and 28% of deaths from pathogens acquired from food each year (Scallan et al., 2011). Globally *Salmonella* ingested through food can be attributed to approximately 80.3 million illnesses and 155, 000 deaths (Majowicz et al., 2010).

Illness from *Salmonella* is traditionally categorized as either typhoidal or nontyphoidal. *Salmonella* Typhi is the most virulent serotype of *Salmonella,* and the cause of typhoid fever, however, *Salmonella* Typhi is only found in humans and not animals (Tortora et al., 2010). Salmonellosis is the general term used to describe illness that occurs as a result of infection with nontyphoidal *Salmonella* (Tortora et al., 2010).

From 1981-2015, outbreaks of salmonellosis globally have been found in a variety of products including juices, chocolate, vegetables, spices, cheese, eggs, fruits and ground meats (Montville et al., 2012). While the known diversity of outbreaks and food products that have been found to maintain and support the survival of *Salmonella* have broadened in recent years, the natural and ubiquitous presence of the bacteria make it a continuing challenge in food production. *Salmonella* is known to be naturally found in the environment and intestinal tracts of humans, poultry and many livestock species and is an understood cause of foodborne gastroenteritis when ingested by humans (Jay et al., 2005; Montville et al., 2012; Tortora et al., 2010). This means that contamination of food, water and the environment with *Salmonella* can usually be traced back to a human or animal source (Grimonnt et al., 2000).

Livestock prevalence

The ability to thrive in a variety of hosts makes *Salmonella* a critical concern with regard to the preparation and processing of animal derived proteins such as meat, milk and eggs. Poultry, cattle, pigs and goats can harbor and shed strains of *Salmonella* without showing any visible indications of illness; thus ultimately leading to illness in humans that have consumed contaminated products (Newell et al., 2010). *Salmonella* commonly live within the digestive tract of many animals, which means unsanitary harvesting conditions could cause food contamination (Tortora et al., 2010).

This can include contamination from fecal matter, ingesta, hair, hide or pelts, and environmental contamination from the processing facility. In order to assess risk of

Salmonella in livestock prevalence studies have been conducted with fecal samples, carcass swabs, hide swabs, lymph nodes and various tissue collections.

The prevalence of *Salmonella* has been shown to vary by season within individual countries. In beef the highest prevalence has been reported in the summer within the U.S (Barkocy-Gallagher et al., 2003; Rivera-Betancourt et al., 2004). In Ireland *Salmonella* prevalence over a one year span was highest from August through September (McEvoy et al., 2003).

Salmonella prevalence on cattle has been found to vary from to 2.2% to 89.6% on hides (Brichta-Harhay et al., 2008; Reid et al., 2002). At pre-evisceration *Salmonella* prevalence has been found at 2.0% to 50.2% for cattle (Brichta-Harhay et al., 2008; Fegan et al., 2005; McEvoy et al., 2003; Rivera-Betancourt et al., 2004). Average fecal shedding of *Salmonella* in cattle has been recorded to range from zero to 66% (Van Donkersgoed et al., 1999; Wells et al., 2001). While extensive research has been conducted about *Salmonella* prevalence in cattle, less has been done about the prevalence of this organism in small-ruminants.

In goats slaughtered in Australia, *Salmonella* was detected in 46.3% of fecal samples, 45.5% of rumen samples and 28.9% of carcass surfaces (Duffy et al., 2009). *S. enterica* subspecies *enterica* serovars were identified in 11.76% of gall bladders and 9.80% of mesenteric lymph nodes from goats harvested in India (Chandra et al., 2006). Another study of sheep in Australia found *Salmonella* present in 5% rumen lymph nodes, 7% abomasal lymph nodes, 7% jejunal lymph nodes, 10% cecal lymph nodes and 7% colonic lymph nodes, while *Salmonella* was detected in 21% of rumen fluid, 17%

14

abomasal contents, 30% Ileum contents, 33% cecum contents and 35% of rectal samples from the same animals (Samuel et al., 1981). A study from the Sudan identified *Salmonella enterica* subspecies *enterica* serotype San Diego in 3.84% of goat mesenteric lymph nodes (El Tom et al., 1999).

A study in Ethiopia found *Salmonella* in 4.8% of fecal samples and 7.7% of mesenteric lymph nodes from small-ruminants (Woldemariam et al., 2005). Additionally tissue samples such as liver, spleen, abdominal and diaphragm muscles from these same animals did not harbor *Salmonella* (Woldemariam et al., 2005). Within central India, fecal, mesenteric lymph nodes, liver and spleen samples yielded a prevalence of *Salmonella* in 3.1% of sheep samples and 3.8% of goat samples, with most positive samples found in the lymph node or fecal samples (Kumar et al., 1973). Sheep carcass surfaces in Switzerland were determined to have *S. enterica* subsp. *diarizonae* in 11% of samples (Zweifel et al., 2004). Researchers in Great Britain only found *Salmonella* present in <1% of sheep fecal samples tested (Davies et al., 2003). A summary of the range of *Salmonella* prevalence determined in small-ruminants can be seen in Table 2.

Role of *Salmonella* in lymph nodes

When attempting to capture the risks associated with a meat product, there are several approaches to the testing and evaluation of the product. For example, understanding what pathogens and organisms are commonly found within the gastrointestinal system or in fecal samples is important because while those systems are removed from the carcass, there is the potential for their contents to get on the carcass

and cause contamination. Being aware of pathogens that could be on the hide or skin of the animal is also important to avoid contamination. The lymphatic system, however, also plays an important role within the living animal, and cannot entirely be removed from the carcass and resulting meat products. As part of the lymphatic system, lymph nodes are an environment where components of the immune system can drain from organs and peripheral tissues providing the opportunity for immune responses as necessary (Worbs & Forster, 2009). The lymphatic system is designed to transport fluids, immune cells and soluble molecules through lymph nodes, which includes foreign substances, inflammatory associated components and anything else within this drainage system (Gashev & Chatterjee, 2013). While lymph nodes generally drain from the organs and systems surrounding them, studies of movement through the drainage system have demonstrated the complexity and therefore inconsistencies, even between nodes located near each other (Soltesz et al., 2006). Translocation, the movement of substances such as microorganisms from the gastrointestinal tract to other nearby organs, such as lymph nodes, has been studied in a variety of animal models (Górski et al., 2006; Steffen & Berg, 1983; Wolochow et al., 1966).

Several studies have reported the prevalence of *Salmonella* found in lymph nodes as a potential source of *Salmonella* contamination in ground meat, especially beef. Mesenteric lymph nodes, the lymph nodes attached to the gastrointestinal tract, are often used as an indicator of overall organism prevalence from the animal. Mesenteric lymph nodes, however, are usually removed as part of the viscera, and therefore not seen as a direct concern for contamination of meat from the carcass (Arthur et al., 2008). Lymph

16

nodes housed within the fatty tissue of the carcass can easily be included as part of the lean and fat trimmings used to make ground meat (Koohmaraie et al., 2012). Therefore, peripheral lymph nodes, such as subiliac lymph nodes, can be considered a more reliable indicator of pathogen contamination risk entering the food supply (Gragg et al., 2013). Mandibular lymph nodes generally filter from salivary glands, the mouth, nose and eyes (Healthline, 2015). Subiliac, mesenteric and mandibular lymph nodes represent three different areas in the carcass, therefore the collection of these three nodes should give a snapshot of overall pathogen prevalence found within the lymphatic system of the animal.

Escherichia coli O157

Overview of organism

Another important member of the *Enterobacteriaceae* family is *E. coli,* a common inhabitant of human and livestock intestinal tracts (Tortora et al., 2010). While generic *E. coli* isn't always threatening, it can have a very serious effect if a pathogenic strain is present. Six groups of *E. coli* are commonly associated with diarrhea and human illness, and they are classified based on their characteristics: enteroaggregative (EAEC) *E. coli,* enterohemorrhagic (EHEC) *E. coli,* enteroinvasive (EIEC) *E. coli,* enteropathogenic (EPEC) *E. coli,* enterotoxigenic (ETEC) *E. coli,* and diffusely adhering *E. coli* (DAEC) (Montville et al., 2012). Furthermore, isolates of *E. coli* can be characterized based on the somatic O antigen, flagellar H antigen and capsule forming K-antigen present on the surface of the organism, with the O antigen used to identify the serogroup of a strain, and

the H antigen an indicator of the serotype (Montville et al., 2012). It should be noted that some of the studies discussed here as well as in the later chapters mention *E. coli* O157:H7, while some just reference *E .coli* O157. The H7 refers to the specific H antigen, and should not be assumed as present even if the colony being cultured behaves as typical *E. coli* O157:H7. Further confirmation beyond basic culturing needs to be done in order to confirm that an isolate is *E. coli* O157 positive and H7 positive before it can accurately be classified as *E. coli* O157:H7 (Feng et al., 2011).

All six of these virulence groups can present a serious health risk to humans, but enterohemorrhagic (EHEC) is especially notable with relation to severe illness. EHEC strains of *E. coli* are also known as Shiga-toxin *E. coli* or STEC, because of the close association between these toxins and the dangerous toxins produced by *Shigella dyseteriae* (Jay et al., 2005; Tortora et al., 2010). The danger with enterohemorrhagic Shiga-toxins (Stx) is the attachment of the pathogen to the endothelial lining of the intestine, which causes hemorrhagic legions, ulcers and irreparable damage (Razzaq, 2015).

Food safety relevance

From exposure to pathogenic *E. coli* to onset of illness symptoms is usually between one and three days (CDC, 2014c). An estimated 2,138 hospitalizations each year can be attributed to illness from foodborne acquired *E. coli* O157:H7, which is roughly 4% of all foodborne illness hospitalizations (CDC, 2014b).

One of the most commonly known EHEC representatives in the U.S., especially with regard to food consumption, is *E. coli* O157:H7. In severe cases *E. coli* O157:H7 symptoms include bloody diarrhea, development of hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) and potentially death ("Control of communicable diseases manual," 2000). Children, elderly and immunocompromised individuals are especially vulnerable to the development of HUS or severe complications as the result of infection with *E. coli* O157:H7. Of patients that develop HUS with diarrheal conditions, 25% of survivors deal with long-term renal complications, and 12% of cases end in death or end-stage renal disease (Garg et al., 2003). Cattle have been attributed as the most important reservoir of *Escherichia coli* O157:H7, and as a potential source of human infection (Pires et al., 2009).

In addition to *E. coli* O157, the FSIS has identified six other STECs that produce Shiga-toxins and possess the intimin gene, which qualify them as adulterants in raw, non-intact beef products: *E. coli* 026, *E. coli* 045, *E. coli* 0103, *E. coli* 0111, *E. coli* 0121 and *E. coli* 0145 (FSIS, 2014a).

Outbreaks, examples

Albert Adam first made one of the first known links between *E. coli,* hemorrhaging of the colon, and kidney failure through an outbreak of infants with bloody diarrhea in 1927 (Noris & Remuzzi, 2005). In 1982, an investigation of an outbreak linked with ground beef served from a restaurant associated with a large incidence gastrointestinal distress and the formation of hemorrhagic colitis was pinpointed to *E.*

coli O157:H7 (Riley et al., 1983). Up until this point in time *E. coli* O157:H7 was not a well-known or notable pathogen of concern in food. Unfortunately, after that outbreak, this pathogen has slowly become more recognizable in the discussion about serious foodborne pathogens. One of the best known outbreaks associated with *E. coli* O157:H7 occurred between November 1992 and February 1993, from undercooked hamburger patties served through at a fast-food establishment chain in the Western U.S. (Tuttle et al., 1999). This outbreak affected over 500 people, with 144 hospitalizations, 30 cases of HUS development and 3 deaths (CDC, 1993). Microbial testing of the lots associated with these illnesses concluded the infectious dose for *E. coli* O157:H7 to be less than 700 organisms (Tuttle et al., 1999). The infectious dose of a microorganism refers to the general minimum number of bacteria needed to cause illness.

Ground beef has been credited with 41% of foodborne *E. coli* O157:H7 outbreaks since 1989, making it the most common food source for this pathogen (Rangel et al., 2005).

Livestock prevalence

In a challenge study conducted in the U.S., sheep continued to shed *E. coli* O157:H7 in their feces for one month post oral inoculation, with the organism found in some tissues of the gastrointestinal tract, but not retained in the contents of the upper gastrointestinal tract (Grauke et al., 2002).

Prevalence of *E. coli* in livestock has been well studied across different regions and seasons, typically by evaluating fecal samples and hide swabs. In the United

Kingdom, one study showed *E. coli* O157 prevalence was 15.7% in cattle, with prevalence ranging by month from 4.8%-36.8% (Chapman et al., 1997). This study also found *E. coli* O157 prevalence in sheep at 2.2%, with the majority of isolates collected between June and September (Chapman et al., 1997). In cattle, *E. coli* O157:H7 prevalence is generally highest during summer months (Van Donkersgoed et al., 1999).

　　E. coli infections from goats or sheep are probably best known for outbreaks from raw milk, or petting zoos. Globally there have been several occurrences of STEC infection resulting in hemolytic-uremia or death linked to raw milk from goat or sheep sources (Bielaszewska et al., 1997; Caro & Garcia-Armesto, 2007; Espié et al., 2006; Stephan, 2008). In 2012, a child developed hemolytic-uremic syndrome (HUS) traced to a visit to a petting zoo with sheep and goats shedding the same strain of Shiga-toxin producing *E. coli* O157:H7 (Heuvelink et al., 2002). In 2014, one death and another serious illness occurred in two children from Oregon, who became infected with *E. coli* O157:H7 that matched the strain found in fecal samples from their pet goat ("Food Safety News", 2014).

　　One study of goats fed varying diets established *E. coli* O157 prevalence as 4.3% from goat fecals, but variations in the diet had no effect on the prevalence (Fox et al., 2007). A comparison of *E. coli* O157:H7 prevalence in sheep found organism detection to be more successful from fecal samples than recto-anal mucosal swabs (McPherson et al., 2015). Over a one month period *E. coli* O157:H7 prevalence varied from 4% - 16% within the same flock of sheep (McPherson et al., 2015).

Campylobacter

Overview of organism

Campylobacter species fall within the *Campylobacteracea* family, as a group of microaerophilic gram negative, non-spore-forming curved rods with livestock, rodents and some domestic pets established as known reservoirs (Montville et al., 2012). *Campylobacter*'s ideal growth temperature is 42°C, which is approximately the temperature animal hosts are able to provide (Tortora et al., 2010). *Campylobacter* spp. are intolerant of drying methods, severely weakened by freezing, and requires a balanced atmospheric environment making it a delicate organism to grow in a laboratory setting (Jay et al., 2005). The ideal gas conditions for *Campylobacter* spp. growth is 85% nitrogen, 10% carbon dioxide and 5% oxygen (FSIS, 2014b).

Food safety relevance

Even with such specific growth requirements, *Campylobacter* presents a serious food safety threat. *Campylobacter* infection is credited as the number one cause of gastroenteritis worldwide, and a leading cause of diarrheal illness (WHO, 2011). It has been estimated that 1.3 million people are affected by campylobacteriosis each year within the U.S. (CDC, 2014a). As reported by the CDC, *Campylobacter* species account for 46% of all laboratory confirmed cases of bacterial gastroenteritis (Altedruse, 1999). Most cases of campylobacteriosis are relatively mild, and self-limiting with diarrhea, fever and gastrointestinal distress lasting from 2-7 days (Jay et al., 2005). Occasionally an

infection with *Campylobacter* can lead to a serious neurological disease with temporary paralysis called Guillain-Barré syndrome (Tortora et al., 2010).

From the laboratory studied isolates of *Campylobacter* species causing illness, *C. jejuni* is responsible for 93% of illnesses, with *C. coli* responsible for the majority of the remaining 7% of illnesses, *C. lari* and *C. fetus* are occasionally identified as well (Gillespie, 2002). With some variation among species, *Campylobacter* has a fairly variable infective dose ranging from 500 organisms to a quantity of 10^6 cells needed to cause illness in the majority of the population (Black et al., 1988; Robinson, 1981).

Livestock prevalence

Campylobacter has long been associated with poultry, because of a high prevalence typically seen in avians (*Campylobacter (3rd Edition)*, 2008). Recently, *Campylobacter* is probably most well-known for causing illness due to the consumption of raw milk. Between 2007 and 2012, *Campylobacter* was the organism responsible for 81% of the 979 human illnesses and 73 hospitalizations that occurred from raw milk outbreaks reported to the CDC (Mungai et al., 2015). But *Campylobacter* spp. have been shown to be shed by pests, poultry, livestock and occasionally even household pets. A study encompassing cases within the U.S. found kennel dogs excrete *Campylobacter* at a rate of 27.3% in their feces, with puppies even more likely to shed the organism than adult dogs (Blaser et al., 1980).

For more than 50 years, *Campylobacter* has been an established veterinary concern, particularly causing abortions in cattle and sheep (Butzler, 2004). Oral ingestion

by the maternal host generally results in an allergic inflammatory response to a

Campylobacter toxin, causing fecal toxicity, hypoxia, sepsis and abortion (Hedstrom et

al., 1987). While known to cause abortions in livestock and occasionally cattle, generally

no outward signs of distress or infection appear in the animal. It is fairly easy for an

animal to be shedding the organism with no detriment to the animal's individual health.

In a large study of *Campylobacter* spp. prevalence in dairy cattle within the U.S., 37.7%

of fecal samples were positive, which represented 80.6% of farm locations visited

(Wesley et al., 2000). A single animal shedding the organism very easily becomes a

potential contaminator of the rest of the farm. For example, one animal shedding the

pathogen in their feces provides opportunity for the pathogen to be carried on the hide of

any other animal in the pen, as well as the potential for other animals to ingest the

pathogen and become reservoirs as well.

   Another possible route of livestock infection or contamination with

Campylobacter studied is the transmission on farms through fly exposure. One such

study in Denmark found 8.2% of flies carrying *C. jejuni* from carrier animals to chickens

housed within a broiler house (Hald et al., 2004). Another study exposed houseflies to *C.*

jejuni and then showed those flies were able to infect previously *Campylobacter* free

avians, with 20% of the flies carrying the organism on their limbs and 70% carried the

organism in their viscera (Shane et al., 1985). In Norway, 50.7% of flies collected on

poultry farms, and 43.2% of flies collected at swine units were carriers of *C. jejuni (*Rosef

& Kapperud, 1983*).*

A study of cattle in Ireland showed *Campylobacter* spp. isolated in 58% of cattle fecal sample, with no influence by transportation in the rate of shedding (Minihan et al., 2004). Within Canada, *Campylobacter* spp. have been identified from 16.9% of pork carcasses, 22.6%-86% of beef carcasses and 43.1% of veal carcasses (Inglis et al., 2004; Lammerding et al., 1988). Identification of *Campylobacter fetus* ssp. *jejuni* (known to cause human gastroenteritis) post-evisceration and final carcass rinsing, was found at a prevalence of 2% in beef carcasses, 24% in sheep and 38% in hogs (Stern, 1981; Svedhem & Kaijser, 1980). Within the U.S., *Campylobacter* shedding in beef has been identified at a rate of 1%-68% with hide contamination between 1% and 25% (Beach et al., 2002).

Almost 60% of cattle shed *Campylobacter* in their feces and milk, but this prevalence doesn't necessarily match retail meat prevalence (Tortora et al., 2010). Retail meat studies have been done over time to assess prevalence of different *Campylobacter* species, as well as some work done with antibiotic resistance of isolates found. *Campylobacter jejuni, C. coli* and *C. lari* isolates were found in U.S. retail chains with the highest prevalence in chicken breast packages (49.9%), ground turkey (1.6%), pork chops (<0.5%) and ground beef (<0.5%) (Zhao et al., 2010). A prevalence study of retail red meat in the United Kingdom found *Campylobacter* in 6.3% of pork, 1.3% of beef, 12.6% of lamb, and 19.8% in "other" meats (described as game animal, rabbit or mutton sources) (Little et al., 2008). In retail meats collected from Turkey, *Campylobacter* spp. were found at 11.1% prevalence in beef, 21.6% prevalence in mutton and 50.4% prevalence in chicken samples (Bostan et al., 2009). A study of retail meats collected in

Iran showed *Campylobacter* spp. in 6% of sheep and 4.4% of goat meat samples collected (Rahimi et al., 2010).

In New Zealand *Campylobacter* was more frequently isolated from sheep offal than beef or pork. *(*Devane, 2005*).* A study of cecum samples from sheep processed in Switzerland found 17.5% prevalence of *Campylobacter* spp. *(*Zweifel et al., 2004*).* Prevalence of *Campylobacter* in Congo was found at 41.2% in raw retail goat meat, 23.7% of ready-to-eat goat skewers and 35.1% in goat fecal samples (a Mpalang et al., 2014). In Greece *Campylobacter* spp. were detected in 63% - 78% of goat carcasses and 72% - 94% of sheep carcasses through a study of several small-ruminant processing facilities (Lazou et al., 2014). In Ethiopia, a study of small-ruminant carcasses found 11% of sheep and 9.4% of goat carcass surfaces positive for *Campylobacter* spp., with the majority of these positive samples found post-evisceration but prior to carcass rinsing (Woldemariam et al., 2009). The higher incidence of contamination found at the post-evisceration step is further indication that carcass contamination is the result of improper hygiene during the evisceration step of harvest. An evaluation of the growth and survivability in sheep fecal samples over time in New Zealand, found *Campylobacter* spp. quickly became inactive in a pasture setting, indicating that infection or contamination of this organism is most likely to occur only in freshly deposited fecal samples (Moriarty et al., 2011). This is not only important to understand for potential risk of infection, but also from a sample analysis standpoint. If *Campylobacter* spp. are easily inactivated over time, on-farm or pasture collected samples may not give a true indication of the actual prevalence of the organism being shed at that location.

Culturing, confirmation and lab techniques

Enrichment media are used to provide the nutrients and conditions for the targeted organism to grow and thrive by reproducing, therefore making it easier to culture and detect if it is, in fact present (Tortora et al., 2010). This is especially helpful when the organism cells are in shock from transport due to a change in their environment. Enrichment can also help improve the ability to culture an organism that is present. Sometimes a selective enrichment is used to suppress other organisms that aren't being targeted, allowing the target organism to have less competition for resources. Selective media provides favorable growth conditions for the target microorganism, while also inhibiting other undesired organisms present in the sample (Montville et al., 2012). Differential media provides physical differences, usually color, to make it easier to identify the target organism (Tortora et al., 2010). With this in mind, appropriate selective and differential media were selected for use in these protocols to maximize accuracy.

For selective enrichment of *Salmonella* in the protocols of this study rappaport-vassiliadis *Salmonella* enrichment (RVS) and tetrathionate (TT) broths were used. RVS selects against other enteric pathogens with malachite green, and it provides the nutrients needed for the enrichment of *Salmonella* (Neogen). The thiosulfate and tetrathionate in TT broth, which forms with the addition of iodine, provide enrichment to *Salmonella* spp. while bile salts inhibit other enteric bacteria from thriving (Neogen). For selective enrichment of *E. coli* O157 in this study, gram negative (GN-VCC) broth with added antibiotics (8 µgml vancomycin, 50 ng/ml of cefixime and 10 µg/ml of cefsulodin) was

used. The GN-VCC broth base is selective for gram-negative organisms, such as *E. coli,* while the antibiotics help suppress other gram negative organisms potentially present in the sample (Neogen).

Xylose lysine deoxycholate (XLD), brilliant green sulfa (BGS) or xylose lysine tergitol (XLT4) agars were used to evaluate growth of *Salmonella*. XLD, is a selective medium for *Salmonella*, by discouraging the growth of gram positive organisms (Himedia, 2015). The addition of sodium thiosulphate and ferric ammonium citrate allows some colonies to produce black centered colonies if capable of fermenting hydrogen sulphide (Himedia, 2015). The formation of this black center makes XLD a differential media as well. BGS agar is also a selective medium used for targeting the growth of *Salmonella* spp. by including ingredients that deter gram positive and other gram negative enteric pathogens (Difco). A pH indicator, phenol red, causes a change in the background also making it easier to differentiate and identify *Salmonella* (Difco). XLT4 has been shown to inhibit *Proteus, Pseudomonas* and many other gram negative, potential enteric pathogens, with hydrogen sulfide production used to differentiate between *Salmonella* and any other organism capable of growing (Remel, 2008). For identification of *E. coli* O157, CHROMagar™ O157 was used, which has been shown to have a 98% accuracy in identification of *E. coli* O157 (Bettelheim, 2002). Chromagar O157 is a selective media that also allows for differentiation of *E. coli* O157 by colony color (Chromogenic media).

For the enrichment of *Campylobacter*, Bolton Broth was used as a selective enrichment with the addition of cefoperazone, vancomycin, trimethoprim and

cycloheximide to suppress unwanted microorganisms from growing (Hunt, 2001).

Modified Charcoal Cefoperazone Deoxycholate Agar (MCCDA) and R&F (RF)

Campylobacter chromogenic plating media were used for growth of *Campylobacter*

(Oxoid)*.* RF has been shown to be more sensitive, and therefore detect *Campylobacter*

jejuni and *Campylobacter coli* at lower quantitates than MCCDA (Gharst et al., 2013).

Immunomagnetic separation (IMS) is a useful laboratory method for the isolation

and culturing of targeted organisms from samples with potentially abundant

microorganisms present (E. Skjerve, 1991). IMS using Dynabeads® Anti-*Salmonella*

selects, separates and concentrates any *Salmonella* found within the sample, even with a

diverse background microflora (Cudjoe et al., 1994). For this reason IMS was

implemented into some of the lab protocols used within this study for both *Salmonella*

and *E. coli* O157.

Considering the number of serovars of *Salmonella* that exist, it is understandable

that it could be difficult to successfully culture all serovars using the same media, and

expect the same typical colonies. Relative to small-ruminants, *S. dublin* and *S. arizonae*

are two serovars studied for their prevalence in sheep, but both are also determined to be

slow in the fermentation of lactose which could make them easy to miss when relying on

this characteristic to identify 'typical' colonies on some media (Wray, 2000).

Agglutination was used as a method to confirm presumptive positive samples, and

decrease the number of falsely recorded positives. Agglutination is an organism specific

lab test that causes a physical change to occur if the antibodies and antigens in the test kit

react with the sample (Tortora et al., 2010). Studies have shown latex agglutination tests

to have up to 100% specificity and sensitivity with confirmation of presumptive positive

E. coli O157:H7 (March & Ratnam, 1989).

Table 1. Host specific *Salmonella* species in livestock

Salmonella species [a]	Livestock species [a]
S. enterica serovar Gallinarum	Poultry
S. enterica serovar Dublin	Cattle
S. enterica serovar Abortus-equi	Horses
S. enterica serovar Abortus-ovis	Sheep
S. enterica serovar Choleraesuis	Swine

a (Jay et al., 2005)

Table 2. Summary of prevalence of Salmonella in small-ruminants

Sample type	Prevalence
Fecal	<1.0% - 46.3% [adf]
Rumen	21.0% - 45.5% [a b]
Abomasum	17% [b]
Rectal	35% [b]
Ileum	30% [b]
Carcass surfaces	11.0% - 28.9% [aei]
Lymph nodes-rumen	5% [b]
Lymph nodes-abomasal	17% [b]
Lymph nodes-cecal	10% [b]
Lymph nodes-colonic	7% [b]
Lymph nodes-mesenteric	3.84% - 14.7% [cdgh]

[a] L. Duffy et al., 2009, [b] Samuel et al., 1981, [c] El Tom et al., 1999, [d] E. Woldemariam et al., 2005, [e] Zweifel et al., 2004, [f] Davies, 2003, [g] Molla, 2006, [h] Nabbut & Al-Nakhli, 1982, [i] Sierra & Gonzalez-Fandos, 1995

Chapter 3: Presence of *Salmonella, Escherichia coli* O157 and *Campylobacter* in small-ruminant (goat and sheep) fecal and hide samples collected in the United States, Bahamas and Mexico

Introduction

According the 2011 CDC estimates, illness acquired from food affect one in six Americans each year, ultimately causing 128,000 hospitalizations and 3,000 deaths annually (CDC, 2014b). The three major foodborne bacterial targets that have had and continue to have the spotlight for research efforts, surveillance and discussion in the food industry relative to products derived from livestock are *Salmonella*, *Campylobacter* and pathogenic *Escherichia coli* (Newell et al., 2010). Nontyphoidal *Salmonella* and *Campylobacter* are both ranked among the top five pathogens responsible for foodborne deaths, hospitalizations and illnesses (CDC, 2014b). Each year in the U.S., an estimated 2,138 hospitalizations can be attributed to foodborne acquired *E. coli* O157:H7 (CDC, 2014b). These three organisms and their targeted research, however, have focused mostly on cattle or pigs, with less application in small-ruminants. Research available about the incidence and impact of *Salmonella* contamination in goat meat is more limited (Duffy et al., 2009). Similarly, while some data exists about the prevalence of *Campylobacter* in many other livestock species, there is very limited published research about the prevalence of this organism in sheep and goats (Horrocks et al., 2009).

An environmental study at an abattoir in Europe found *E. coli* O157 in 2.2%, *Salmonella* in 1.1% and *Campylobacter* in 5.6% of sheep holding pens (Small et al., 2002). Within the U.S. *E. coli* O157:H7 was identified on 12.8% and *Salmonella* on

14.4% of lamb hides from commercial abattoirs (Kalchayanand et al., 2007). Small

abattoirs in Nigeria had *E. coli* O157:H7 in 2.5% of sheep hide swabs and 10% of fecal

samples, while goat fecal samples had a prevalence of 5% and no *E. coli* O157:H7 was

detected on the hides (Akanbi & Mbah, 2011). In Ireland, *E. coli* O157:H7 prevalence

was 5.75% in fleece samples but undetected in fecal samples (Lenahan et al., 2007).

Outbreaks of *E. coli* infection associated with sheep or goats have mostly been

linked to raw milk, cheese or petting zoos (Bielaszewska et al., 1997; Caro & Garcia-

Armesto, 2007; Espié et al., 2006; Stephan et al., 2008). Considering the potential

diversity of products derived from small-ruminants and their role in the global market,

understanding potential pathogens associated with their production is crucial. The

objective of this study was to evaluate the presence of *Salmonella* and *E. coli* O157 found

on the hides of sheep and goats, as well as identifying presence of *Salmonella,*

Campylobacter and *E. coli* O157 found in small-ruminant fecals.

Materials and methods

Sample collection

Hide samples were collected from sheep and goats at small (1-30 animals per day)

and large (800-1,000 animals per day) sized abattoirs located in California ($n = 62$), New

Mexico ($n = 45$) and Texas (*Salmonella $n = 338$*; *E. coli* O157 $n = 266$) over a 14-month

period (

Table **3**). Hides were swabbed after exsanguination, but prior to hide removal, using a sterile cellulose sponge, pre-moistened with 25 mL of buffered peptone water (BPW, World Bioproducts; Mundelein, Illinois) in an approximately 100 cm^2 area at four locations per each carcass (leg, midline, foreshank, breast/neck) to collect a sample representative of the hide surface. Samples were immediately placed in plastic, insulated coolers kept cold ($\leq 4°C$) using previously frozen ice packs. Coolers containing samples were transported to the laboratories at Texas Tech University in Lubbock, Texas, or Angelo State University in San Angelo, Texas for processing.

Small-ruminant fecal samples were obtained from abattoirs located in California, Texas and New Mexico after evisceration by cutting the lower part of the colon, closest to the bung and rectum, with a clean knife and guiding 10-15 grams of fecal pellets from the colon into a sterile collection cup. If 10-15 grams were not available at the end of the colon, all available sample was collected, no sample from another part of the gastrointestinal tract was collected to make up the difference. Farm fecal samples were also collected from sheep and goat farms in the Bahamas, Mexico, California and Texas. Fresh, sterile gloves were used to pick up farm fecals from pens where sheep or goats had been present within 12 hours of sample collection, with care being made to collect the most freshly deposited samples possible. Gloves were changed before each new collection was gathered, samples were immediately deposited into a sterile plastic collection cups or whirlpak™ bag (Nasco; Fort Atkinson Wisconsin). A minimum of three animals were in each pen per every one fecal sample collected. Some farms sampled had sheep and goats living together in the same pens, making it impossible to

distinguish which specie a fecal sample originated from. These samples were collected and recorded in the lab notebook as mixed specie farms. The analysis of these samples was not categorized as either goat or lamb, but instead kept as an independent group of the species designation, and included in the overall analysis for each organism when not differentiated by specie.

Samples were immediately placed in plastic, insulated coolers kept cold (≤ 4°C) using previously frozen ice packs. Coolers containing samples were transported to the laboratories at Texas Tech University in Lubbock, Texas, California State University Fresno in Fresno, California or Angelo State University in San Angelo, Texas for processing. A United States veterinary permit for importation and transportation of controlled materials and organisms and vectors (USDA-APHIS research permit # 114031) was obtained, which granted permission for the transportation of fecal samples from the Bahamas and Mexico to the U.S. for analysis.

Hide swab sample processing

After arrival to the laboratory, hide swabs in BPW were homogenized for 30 seconds at 230 RPM using an automated stomacher (Steward Laboratory Systems; Davie, FL) to ensure adequate distribution of microorganisms within the sample bag. *Salmonella* analysis was done as described in the FDA Bacteriological Analytical Manual (FDA BAM; 2014). One mL of the sample was added to nine mL Rappaport-Vassiliadis broth (RV; Oxoid, Hampshire, UK), and nine mL of Tetrathionate broth with iodine (TT; Neogen; Lansing, MI). Enrichments were incubated at 42°C for 18-24 hours. TT and RV

enrichments were streaked to Xylose-Lysine-Tergitol agar (XLT4; Hardy diagnostics; Santa Maria, CA) and incubated 18-24 hours at 37°C. Presumptive positive *Salmonella* colonies identified by phenotypical characteristics on XLT4 agar (yellow or red colonies with black centers) were subjected to latex agglutination (Oxoid; Hampshire, UK) for confirmation using manufacturer's instructions. Isolates of agglutination positive colonies were selected for further enrichment into TSB for 24 hours at 37°C, and frozen in duplicate with 20% glycerol for further analysis at a later time.

For detection of *E. coli* O157 in the hide samples, one mL of sample was added to nine mL of Gram-Negative broth with 8 µgml vancomycin, 50 ng/ml of cefixime and 10 µg/ml of cefsulodin (GN-VCC; Hardy Diagnostics; Santa Maria, CA) and incubated at 37°C for six hours (Echeverry et al., 2006). Enriched GN-VCC was then subjected to Immunomagnetic Separation techniques (IMS) using anti-*E.coli* O157 Dynabeads® (Invitrogen; Grand Island, New York). IMS product was then plated onto CHROMagar™ O157 with 2.5mg/L tellurite (Chromagar; Springfield, NJ) and incubated 24 hours at 37°C. Phenotypically presumptive positive colonies were identified by their mauve color as described with the CHROMagar™ O157 media guidelines (Chromagar; Springfield, NJ). These presumptive positive colonies were recorded in a lab notebook and confirmed using latex agglutination (DrySpot™ *E. coli* O157 Latex Agglutination Test, Thermo Scientific; Lenexa, KS). Isolates of agglutination positive colonies were selected for further enrichment into TSB for 24 hours at 37°C, and frozen in duplicate with 20% glycerol for further analysis at a later time.

Fecal sample processing

For *Salmonella* analysis, one gram of each fecal sample was added to nine mL of TT broth, and one gram added to nine mL of RV broth, and incubated at 42°C for 18-24 hours. Procedure for detection, confirmation and freezing of isolates was the same as hide sample processing for *Salmonella.*

For detection of *E. coli* O157, one gram of fecal sample was added to nine mL of GN-VCC. The enrichment, culturing, confirmation and freezing of isolates from fecals followed the same protocol as hide samples for *E. coli* O157.

For *Campylobacter* analysis, a variation of the USDA FSIS Microbiological Laboratory Guidelines for isolation and identification of *Campylobacter jejuni/coli/lari* from poultry products was used (FSIS, 2014). Five grams of fecal sample was added to a filtered whirlpak™ bag (Nasco; Fort Atkinson Wisconsin) with 30 mL of Bolton broth with selective supplement (EMD Chemicals Inc.; Gibbstown, NJ). Bags were homogenized for 30 seconds at 230 RMP using an automated stomacher. Then, 30 mL of the Bolton broth homogenate was transferred into plastic flasks with filtered screw cap lids (Nunc® EasYFlasks™, Sigma-Aldrich; St. Louis, MO). The samples were then incubated under microaerophilic (85% nitrogen, 10% carbon dioxide and 5% oxygen) conditions (GasPak™ EZ Campy Container system sachets, Becton, Dickinson and Company; Sparks, MD) using sealed anaerobic chambers for 48 hours at 37°C. After incubation, samples were streaked from Bolton broth to Modified Charcoal Cefoperazone Deoxycholate agar (MCCDA, Oxoid Company; Cambridge, UK) agar plates and R&F® *Campylobacter* chromogenic plating media (R&F, R&F labs; Chicago, IL). Plates were

incubated in microaerophilic (85% nitrogen, 10% carbon dioxide and 5% oxygen) chambers for 60 hours. Following incubation, typical colonies were subjected to latex agglutination (*Campylobacter* latex agglutination kit, Oxoid; Hampshire, UK). Positive colonies were inoculated into 5 mL tubes of Bolton broth with selective enrichment, grown in microaerophilic environments for 48 hours, mixed with glycerol and frozen for future analysis.

Statistical analysis

Prevalence percentages were calculated using the frequency procedure of SAS (SAS Institute Inc., Cary, NC). Hide swabs were analyzed for each organism (*Salmonella* and *E. coli* O157). Frequency was calculated controlling for lamb and goat species, as well as combined frequencies. For fecal samples, frequency of positive samples for each organism (*Salmonella*, *Campylobacter* and *E. coli* O157*)* was calculated controlling for species, as well as calculated frequency for sheep, goats and mixed pens to create an overall presence. Significance for all calculations was detected at $P \leq 0.05$. Chi-square comparisons were made between species.

Results

Presence of *Salmonella* was detected at 10.33% in goats and 11.41% in lamb fecal samples. *E. coli* O157 presence was 19.69% in goats and 9.83% in lamb fecals. *Campylobacter* was identified in 70.97% of goat and 75.00% of lamb samples. Fecals collected from mixed pens containing sheep and goats living together had 42.0%

Salmonella, 24.00% *E. coli* O157 and 97.96% *Campylobacter* presence. Overall, small-ruminant fecal samples, were found to have a presence of 13.91% *Salmonella* (N=532)*,* 15.30% *E. coli* O157 (N=477) and 80.68% *Campylobacter* (N=176)*.* Frequency of *Salmonella, E. coli* O157 and *Campylobacter* by specie and organism can be seen in Table 4. Frequency of *Salmonella, E. coli* O157 and *Campylobacter* in small-ruminant fecal samples organized by month are shown in Table 6. Of all the sheep, goat and mixed pen fecal samples analyzed, 14 samples were positive for *Salmonella* and *E. coli* O157, 28 samples were positive for both *Salmonella* and *Campylobacter,* 28 samples were positive for *E. coli* O157 and *Campylobacter* and 11 samples were found to be positive for all three organisms.

Overall combined hide prevalence of *Salmonella* found in small-ruminants, goats and lambs, was 17.11%. *Salmonella* presence was detected in 3.31% of goat hide samples and 25.47% of lamb hide samples. *E. coli* O157 was present in 1.65% of goat hide samples and 1.38% in lamb hide samples. Total presence of *E. coli* O157 for sheep and goat hides combined was 1.50% (Table 7). Sources of hide samples collected across states, months and species are detailed in

Table 9. *E. coli* O157 hide prevalence overall for small-ruminants (sheep and goats combined) was significantly lower than fecal prevalence (*P* < .001). *Salmonella* hide and fecal relationship was not significant (*P* = 0.215).

While the objective of this study was not to specifically target show animals, a portion of the hide and fecal samples collected came from animals that previously had

been raised and exhibited for livestock exhibition. For show goats and lambs significant

differences between fecal and hide prevalence of both *Salmonella* ($P =0.006$) and *E. coli*

O157 ($P <0.001$) were detected (Table 10).

Table 3. Description of sources for fecal samples collected from sheep and goats by sampling region, month and year, facility type, species and quantity analyzed for *Salmonella, E. coli* O157 and *Campylobacter*

Location	Month and year of collection	Farm or plant	Species	# of samples analyzed for each organism		
				Salmonella	*E. coli* O157	*Campylobacter*
Bahamas	July 2014	Farm	Goats	28	28	n/a
	March 2015	Farm	Goats	50	50	49
Mexico	April 2014	Farm	Goat	25	28	n/a
California	April 2015	Plant	Goat	43	43	17
	April 2015	Plant	Lamb	15	15	10
	April 2015	Farm	Goat	14	14	14
	April 2015	Farm	Lamb	26	26	25
New Mexico	November 2014	Plant	Lamb	45	45	n/a
Texas	February 2014	Plant	Goat	24	24	n/a
	April 2014	Plant	Lamb	14	14	n/a
	July 2014	Plant	Lamb	1	2	n/a
	July 2014	Plant	Goat	5	10	n/a
	August 2014	Plant	Goat	2	4	n/a
	September 2014	Plant	Lamb	12	n/a	n/a
	October 2014	Plant	Goat	8	8	n/a
	October 2014	Plant	Lamb	18	10	n/a
	November 2014	Plant	Lamb	40	n/a	n/a
	January 2015	Plant	Lamb	5	n/a	n/a
	February 2015	Plant	Lamb	19	18	n/a
	February 2015	Farm	Lamb	61	61	61
	March 2015	Plant	Lamb	45	45	n/a
	March 2015	Plant	Goat	26	26	n/a
	April 2015	Plant	Lamb	6	6	n/a
			Total	532	477	176

Table 4. Presence of *Salmonella, E. coli* O157 and *Campylobacter* in fecal samples from small-ruminants in the Bahamas, U.S. and Mexico

Organism	Specie	# positive	Total Samples	Presence
Salmonella	Goats	19	184	10.33%
	Lambs	34	298	11.41%
	Mixed pens[a]	21	50	42.0%
	Total	74	532	13.91%
E. coli O157	Goats	38	193	19.69%
	Lambs	23	234	9.83%
	Mixed pens[a]	12	50	24.00%
	Total	73	477	15.30%
Campylobacter	Goats	22	31	70.97%
	Lambs	72	96	75.00%
	Mixed pens[a]	48	49	97.96%
	Total	142	176	80.68%

[a] Mixed pens includes samples from collected at farms that had sheep and goats living together in the same pens

Table 5. Fecal sample, by region of collection (state or country), presence of *Salmonella*, *E. coli* O157 and *Campylobacter* from small-ruminants in the Bahamas, U.S. and Mexico

	Salmonella		*E. coli* O157		*Campylobacter*	
Location	N	Presence	N	Presence	N	Presence
Bahamas	78	41.03%	78	19.23%	49	97.96%
Mexico	25	20.00%	28	0.0%	-	-
California	98	0.0%	98	19.39%	66	77.27%
New Mexico	45	8.89%	45	0.0%	-	-
Texas	286	11.54%	228	17.11%	61	70.49%
U.S. total	429	8.62%	371	15.63%	127	74.02%
Total (all locations)	532	13.91%	477	15.30%	176	80.68%

Table 6. Fecal samples by month of collection presence of *Salmonella, E. coli* O157 and *Campylobacter* from small-ruminants in the U.S., Bahamas and Mexico

Month	*Salmonella*		*E. coli* O157		*Campylobacter*	
	# Pos/ Total	%	# Pos/ Total	%	# Pos/ Total	%
January	4/5	80.00%	-	-	-	-
February	5/102	4.67%	19/103	18.45%	43/61	70.49%
March	31/121	25.62%	19/121	15.70%	48/49	97.96
April	7/143	4.90%	27/146	18.49%	51/66	77.27%
July	12/34	35.29%	3/40	7.50%	-	-
September	2/12	16.67%	-	-	-	-
October	4/28	14.29%	5/18	27.78%	-	-
November	9/82	10.98%	0/45	0.0%	-	-

Table 7. Presence of *Salmonella* and *E. coli* O157 in hide samples from small-ruminants in California, New Mexico and Texas

Organism	Specie	# positive	Total Samples	Presence
Salmonella	Goats	4	121	3.31%
	Lambs	54	212	25.47%
	Total	58	339	17.11%
E. coli O157	Goats	2	121	1.65%
	Lambs	2	145	1.38%
	Total	4	266	1.50%

Table 8. Hide samples designated by month presence of *Salmonella* and *E. coli* O157

Month	*Salmonella*		*E. coli* O157	
	# Pos/ Total	%	# Pos/ Total	%
January	4/5	80.00%	-	-
February	2/46	4.35%	0/42	0.0%
March	12/71	16.90%	2/71	2.82%
April	5/77	6.10%	1/82	1.22%
July	0/6	0.0%	0/6	0.0%
August	-	-	0/2	0.0%
September	0/12	0.0%	-	-
October	3/28	14.29%	1/18	5.56%
November	32/83	38.55%	0/45	0.0%

Table 9. Total hide samples analyzed for *Salmonella* and *E. coli* O157 detailed by species, location and month and year of collection

Location	Month and year of collection	Species	# of samples analyzed for each organism	
			Salmonella	*E. coli* O157
California	April 2015	Goat	47	47
	April 2015	Lamb	15	15
New Mexico	November 2014	Lamb	45	45
Texas	March 2014	Goat	24	24
	April 2014	Lamb	14	14
	July 2014	Goat	5	5
	July 2014	Lamb	1	1
	August 2014	Goat	1	1
	August 2014	Lamb	1	1
	September 2014	Lamb	12	n/a
	October 2014	Goat	8	8
	October 2014	Lamb	8	n/a
	October 2014	Lamb	10	10
	November 2014	Lamb	39	n/a
	November 2014	Goat	4	n/a
	January 2015	Lamb	5	n/a
	February 2015	Lamb	12	8
	February 2015	Goat	10	10
	March 2015	Lamb	10	10
	March 2015	Goat	26	26
	March 2015	Lamb	35	35
	April 2015	Lamb	6	6
		Total	338	266

Table 10. Show sheep and goats from Texas presence of Salmonella and E. coli O157 in fecal and hide samples

Species	Fecal		Hide			Fecal		Hide		
	n	%	*n*	%	*P*-value	*n*	%	*n*	%	*P*-value
Goats	58	**3.45%**	58	**6.90%**		58	**37.93%**	58	**3.45%**	
Lambs	59	**6.78%**	59	**16.95%**		45	**4.44%**	45	**0.00%**	
Total	117	**5.13%**	117	**11.97%**	*P* = 0.006	103	**23.30%**	103	**1.94%**	*P* < 0.001

Discussion

Our data indicate that *Salmonella, Campylobacter* and *E. coli* O157 are present in small-ruminants. Comparing *E. coli* O157 presence between goat and lamb hides was very similar at 1.57% and 1.38% respectively. The significantly lower presence ($P <$ 0.0001) of *E. coli* O157 from hide samples (1.47%) when compared to presence in fecal samples (14.02%), is not consistent with trends expected. In cattle, hides have sometimes been shown to carry *E. coli* O157 at a higher frequency than fecal prevalence from pens of the same animals, often explained by the concept of super-shedder cattle (Arthur et al., 2009). Quantified as $\geq 10^4$ colony-forming units/g of *E. coli* O157:H7 in feces, super-shedder cattle, refers to the idea that some animals in the population shed the organism at a much higher rate than the majority of the other animals in the group (Munns et al., 2015). Our study did not quantify organism presence at any point in time, since the objective was just to look for presence or absence of each respective organism. Potentially the quantity of those organisms present in small-ruminants are lower in concentration than the quantity seen in cattle, therefore decreasing the likelihood of super-shedders having an effect on the hide of other animals within the same pen. In the Scottish cattle population, 20% of the most highly infected animals were responsible for approximately 80% of *E. coli* O157 found in fecal samples from their pens (Matthews et al., 2006). As livestock are often worked through confined pens, transported and comingled with new animals all prior to processing, the idea that a super-shedder could have a significant impact on environmental contamination and subsequently hide contamination of other animals is important to consider (Arthur et al., 2010). For

example, in the U.S., a 9-month study found that cattle pens where *E. coli* O157

prevalence was higher than 20% in fecal samples, the cattle had a hide prevalence of 80%

or higher (Arthur et al., 2009).

In the U.S., *E. coli* O157:H7 prevalence in feedlot lambs was found to be 9% in

fecal and 18% in wool samples (Edrington et al., 2009). In Ireland, 5.75% of fleece

samples and none of the fecal samples cultured, from the same animals, had *E. coli* O157

(Lenahan et al., 2007). In Ethiopia, *E. coli* O157:H7 prevalence in goats was 3.3% from

fecals and 10% from hides, in sheep 5.4% fecal prevalence and 8.0% hides (Mersha et al.,

2010).

However, higher prevalence in hides than fecal samples is not always the

observation. In one example, cattle fecal prevalence was 28% and hide prevalence of *E.

coli* O157 was 11% (Elder et al., 2000). This lower hide prevalence was attributed to the

limited sampling location used to represent the hide (Elder et al., 2000). In our study, the

sampling locations of the hide were based on the published finding that the foreshank,

hindshank and leg have been shown to represent the highest prevalence of *E. coli*

O157:H7 on a beef carcass (Rekow et al., 2010). Therefore, the protocols for this study

were designed so that four areas of the hide were swabbed with the same sponge in order

to get a better representation of pathogen presence.

One of the potential explanations for the lower than expected hide prevalence

could be the fiber length and type of the animals in this study. Goats and sheep can have

very different fiber types. Some of the lambs used in this study were hair sheep breeds,

and nearly half of the lambs and goats were former show animals. The frequency of

47

Salmonella detected on show animal hides was 11.97%, while only 5.13% of fecals from these same show animals were *Salmonella* positive. *E. coli* O157 was present in 23.30% of the fecals from show animals, while only present on 1.94% of hides from show animals. There was significant difference in the presence on hides and in fecals within each respective organism, *Salmonella* (P=0.006 and *E. coli* O157 ($P < 0.001$). The majority of show animals within this study were only analyzed for *Salmonella* and *E. coli* O157, so *Campylobacter* was not evaluated for presence in show animals. These animals may have been on a more intensive feed program and had more opportunity for environmental pathogen exposure. But these former show animals had clipped or shorn coats at the time of harvest, and likely had been exposed to frequent washings during their show careers. The shorn lambs and clipped goats had less length of coat and therefore, potentially less opportunity for fecal or pathogen attachment. In Norway unshorn lamb carcasses had a significantly higher frequency of *E. coli* found then carcasses of shorn lambs (Hauge et al., 2011).

A study of different sampling methods to assess pathogen presence on cattle hides found that using clipped hair samples allowed the highest recovery of microorganisms, followed swabbing of the area (Reid et al., 2002). Recovery of pathogens from the hide samples was not inadequate, however. *Salmonella* prevalence was 17.11% for small-ruminants combined for this study. Granted, lamb hide prevalence was numerically much higher than goats, these positive samples were not simply the reflection of one abattoir or cohort of animals. The lamb hides found positive for *Salmonella* were distributed across time-points and sampling locations. The prevalence of *Salmonella* found in small-

ruminant hides is comparable to the range previously found. Feedlot sheep in the U.S. from one study had *Salmonella* in 50% of wool samples, but only 7% of fecal samples (Edrington et al., 2009). Another study of commercial abattoirs in the U.S. found *Salmonella* on 14.4% of lamb hides (Kalchayanand et al., 2007). Sheep hides at an abattoir in Europe found a prevalence of 5.5% *E. coli* O157, 7.8% *Salmonella* and zero *Campylobacter* positives (Small et al., 2002). Sampling done on lamb hides from commercial abattoirs in the U.S. found 12.8% *E. coli* O157:H7 prevalence and 86.2% non-O157 STEC (Kalchayanand et al., 2007).

Lab methods used for detection of pathogen prevalence from samples with a potentially diverse background of microflora is important. Immunomagnetic separation (IMS) is one method useful for the isolation and culturing of targeted organisms from samples with potentially cluttered background microflora (Skjerve, 1991). For example, performing IMS using Dynabeads® Anti-Salmonella selects, separates and concentrates any *Salmonella* found within the sample, even with a diverse background microflora (Cudjoe et al., 1994). The use of the IMS procedures have been shown to allow detection with as low as 1-5 cells/25 g of sample (Cudjoe et al., 1997). IMS procedures were used in this study for the hide and fecal samples for *E. coli* O157 and *Salmonella* to identify the targeted organisms if they were in fact present.

The high prevalence of *Campylobacter* (80.68%) detected from the fecal samples in this study is concerning. This is higher than most of the previous reports found. In New Zealand, *Campylobacter* was more frequently isolated from sheep offal than beef or pork (Devane, 2005). A study of samples from the cecum of sheep processed in Switzerland

49

found 17.5% prevalence of *Campylobacter* spp. (Zweifel et al., 2004). Prevalence of *Campylobacter* in Congo was found at 41.2% in raw retail goat meat, 23.7% of ready-to-eat goat skewers and 35.1% in goat fecal samples (a Mpalang et al., 2014). In Greece *Campylobacter* spp. were detected in 47%-67% of goat carcasses and 62%-86% of sheep carcasses through a study of several small-ruminant processing facilities (Lazou et al., 2014). In Ethiopia, a study of small-ruminant carcasses found 11% of sheep and 9.4% of goat carcass surfaces positive for *Campylobacter* spp., with the majority of these positive samples found post-evisceration but prior to carcass rinsing, indication of contamination during processing (Woldemariam et al., 2009).

A comparison of *E. coli* O157 prevalence in sheep found organism detection to be more successful from fecal samples than recto-anal mucosal swabs (McPherson et al., 2015). Because the objective of this study was to determine overall pathogen presence, lab methods were used to target the most successful recovery of organisms if they did exist in the hide or fecal samples. An evaluation of the growth and survivability in sheep fecal samples over time in New Zealand, found *Campylobacter* spp. quickly become inactive in a pasture setting, indicating that infection or contamination of this organism is most likely to occur only in freshly deposited fecal samples (Moriarty et al., 2011). Considering that many of these samples were collected from farms, the potential prevalence of *Campylobacter* in the previous studies could have been even higher than successfully cultured in the lab.

Conclusion

These results confirm the presence of *Salmonella, E. coli* O157 and *Campylobacter* in small-ruminants. The high prevalence of *Campylobacter* found in fecal samples could be concerning if care is not taken to avoid contamination and spreading of this pathogen during processing and handling methods. In addition, the presence of this organism can be a concern for contact between humans and small-ruminant fecal samples, through close living quarters, petting zoos or unhygienic management conditions. The low hide prevalence for *E. coli* O157 compared to fecal prevalence is of interest for further investigation. Further work should be done to examine effect of hair or fiber type on pathogen prevalence. This could potentially be done by looking at hair breeds, fiber breeds, effect of wool length and coat cleanliness evaluation along with sample collection. Additional work could be done to look at effect of season or geography on the prevalence of the three respective organisms. None of the samples collected in this study were tested for virulence, however further understanding of virulence could be an indicator of the potential risk of illness from these pathogens.

Chapter 4: Presence of *Salmonella* on carcass surface and retail small-ruminant (sheep and goat) samples collected in the United States and Bahamas

Introduction

In 2013, an estimated 13.9 million tons of lamb were produced, a slight increase from lamb production globally in 2012 (FAO, 2014b). While compared to beef, pork or poultry consumption this number may seem insignificant, small-ruminants are still an important and valued protein source. Globally, per capita consumption of lamb, goat and mutton was 4.17 pounds in 2007, an increase from 3.95 in 1965 (Brester, 2012). The U.S. annual consumption is less than a pound per person, compared with 5.5 pounds per capita in Africa, and 26 pounds in Australia (Brester, 2012).

Salmonella are estimated to cause 11% of total foodborne illness, 30% of foodborne hospitalizations and 28% of deaths from pathogens acquired from food each year in the U.S. (Scallan et al., 2011). Globally *Salmonella* ingested through food can be attributed to approximately 80.3 million illnesses and 155, 000 deaths (Majowicz et al., 2010). Poultry, cattle, pigs and goats can harbor and shed strains of *Salmonella* without showing any visible indications of illness; this ultimately leading to illness in humans that have consumed products from contaminated products (Newell et al., 2010). *Salmonella* commonly live within the digestive tract of many animals, which means unsanitary harvesting conditions could cause meat contamination (Tortora et al., 2010).

Research has shown *Salmonella* prevalence in beef carcasses normally ranges

from 7.6% - 58% (Bosilevac et al., 2009; Fluckey et al., 2007; Loneragan et al., 2012;

McEvoy et al., 2003; Maradiaga et al., 2014; Rivera-Betancourt et al., 2004). Post-

intervention *Salmonella* prevalence in the U.S. has been shown to range from zero to

1.3% (Bacon et al., 2002; Narvez-Bravo et al., 2012; Rivera-Betancourt et al., 2004). On

the other hand, data is more limited on *Salmonella* presence in small-ruminants. In Spain,

Salmonella prevalence on lamb carcasses was identified at 10% (Sierra & E. Gonzalez-

Fandos, 1995). Lamb carcasses from the U.S. were shown were shown to have a

Salmonella prevalence of 1.9% in the fall/winter months and 1.2% in the spring (Duffy &

Belk, 2001). A study of Australian lamb retail samples determined *Salmonella* prevalence

to be 1.3% in bone-less meat samples. The objective of this study was to identify

Salmonella presence in carcasses as well as identification of *Salmonella* in available retail

meat from goat and lambs in the United States.

Materials and methods

Sample collection

Carcass surface samples were collected at small (1-30 animals harvest per day)

and large (800-1,000 animals per day) sized abattoirs located in California, New Mexico

and Texas over a 14-month period. Each abattoir location was represented by a minimum

of three sampling days to attempt to have accurate representation of organism presence.

Carcasses were sampled using sterile cellulose sponges, pre-moistened with 25 mL of

buffered peptone water (BPW; World Bioproducts; Mundelein, Illinois). For each

carcass, at each time-point, one sponge was used to swab approximately 100 cm^2 at four locations (leg, midline, foreshank and breast/neck) to collect a sample representative of the carcass surface at that time-point (Figure 1.). The three time-points used in this study were: pre-evisceration, post-evisceration and post-intervention. Attempts were made to collect carcass swab samples at the three time-points from the same animal, however, this was not always achieved due to occasional adjustments made by plant personnel in the commercial processing facility in California.

Retail samples were collected in the Bahamas, Texas and California from local establishments. Packaging type and origin of meat were not used as criteria in selecting these samples, and therefore were not used in analysis. In the Bahamas, many retail meat samples were labeled as mutton, and understood to refer to either sheep or goat meat from animals over one year but not differentiating which small-ruminant species (M. Messina, personal communication, April, 2014). Whole muscle retail samples were swabbed using sterile cellulose sponges, pre-moistened with 25 mL of buffered peptone water (BPW; World Bioproducts; Mundelein, Illinois). Both sides of the muscle as well as any purge present in the package was swabbed to represent the sample. Non-frozen retail samples were targeted in this study; however, if only frozen product was available it was allowed to thaw in refrigeration (4°C) before swabbing.

Samples were immediately placed in plastic, insulated coolers kept cold ($\leq$ 4°C) using previously frozen ice packs. Coolers containing samples were transported to the laboratories at Texas Tech University in Lubbock, Texas, California State University, Fresno in Fresno, CA or Angelo State University in San Angelo, Texas for processing. A

United States veterinary permit for importation and transportation of controlled materials

and organisms and vectors (USDA-APHIS research permit # 114031) was obtained,

which granted permission for the transportation of retail samples from the Bahamas to the

U.S. for analysis.
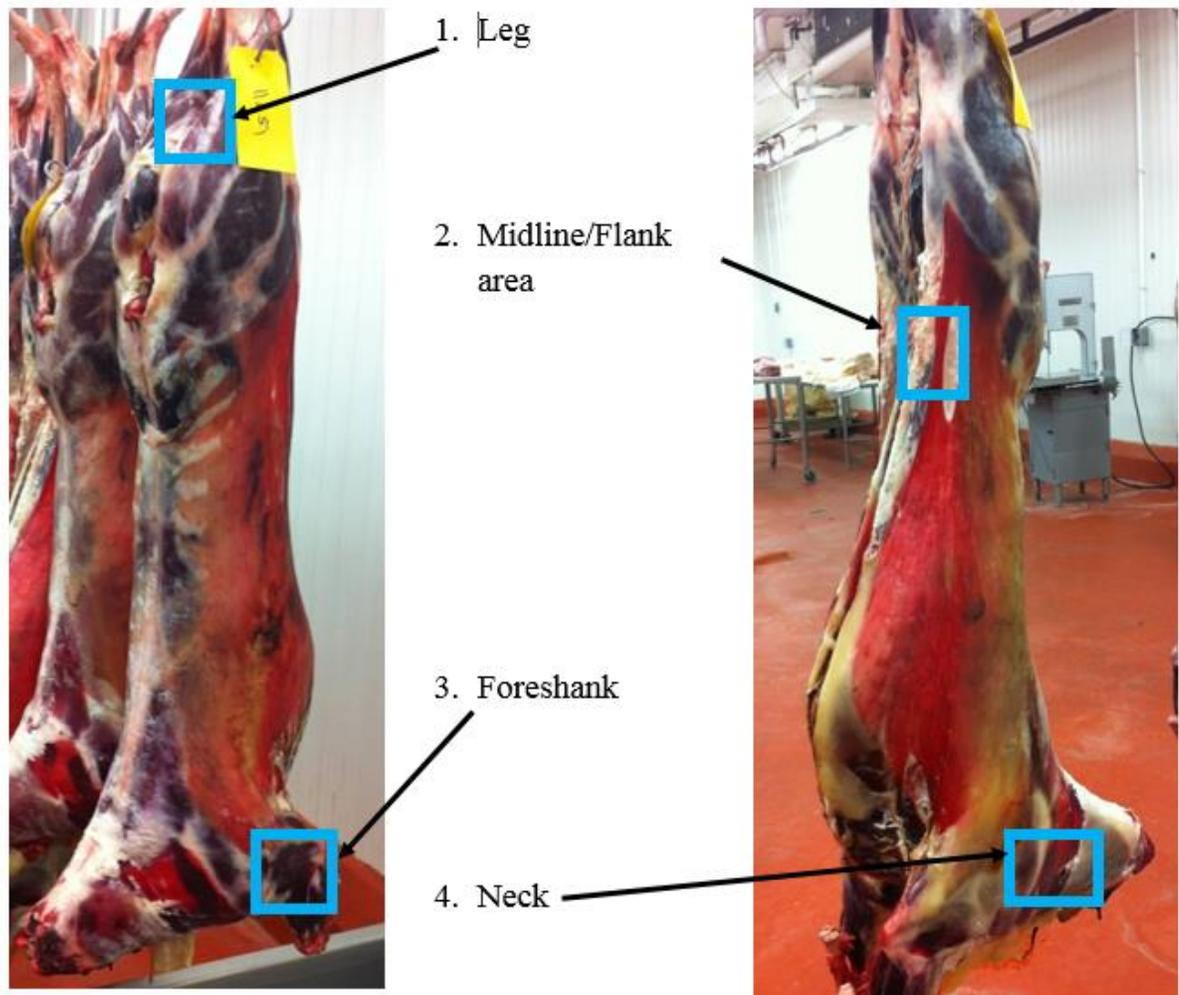
Figure 1. Four locations sampled on the carcass surface to create a composite carcass surface swab for each time-point

Sample processing

All carcass and retail samples were processed using the same laboratory methods. After arrival to the laboratory, sponges in BPW were homogenized using an automated stomacher (Steward Laboratory Systems; Davie, FL) for 30 seconds at 230 RPM. One mL of sample was added to nine mL of Tryptic Soy Broth (TSB; Becton, Dickinson and Company; Franklin Lakes, NJ) and incubated 18-24 hours at 42°C. Samples were then prepared and run using the GeneDisc® Cycler (Pall Corporation; Port Washington, New York) for *Salmonella* detection. GeneDisc® is a real time PCR system that allows for the detection of the targeted organism using a provided master-mix. Plates can either be run with six or twelve samples at a time.

Presumptive positive samples from the GeneDisc® results were selected from original TSB enrichment broth, one mL added to nine mL of Tetrathionate broth with iodine (TT; Neogen; Lansing, MI) and nine mL of Rappaport-Vassiliadis broth (RV; Oxoid; Hampshire UK), incubated 24 hours at 42°C, and then streaked to xylose lysine deoxycholate (XLD; Hardy-Diagnostics; Santa Maria, CA) and brilliant green sulfa (BGS; Remel; Lenexa, KS) agar plates. BGS and XLD plates were incubated 18-20 hours at 37°C.

BGS plates were evaluated for pink or opaque colonies surrounded by a red zone, indication of presumptive positive *Salmonella* as indicated by the FSIS MLG (FSI, 2014). XLD plates were evaluated for red or pink colonies with a black center, as described by the media manufacturer (Hardy Diagnostics). Presumptive positive *Salmonella* colonies from BGS and XLD plates were subjected to latex agglutination (Oxoid; Hampshire, UK)

for confirmation using manufacturer's instructions. Isolates of agglutination positive colonies were selected for further enrichment into TSB for 24 hours at 37°C, and frozen in duplicate with 20% glycerol for further analysis at a later time.

Statistical Analysis

Prevalence percentages were calculated using the frequency procedure of SAS (SAS Institute Inc.; Cary, NC). For carcass swabs, frequency was calculated for each respective time-point (pre-evisceration, post-evisceration and post-intervention) controlling for lamb and goat carcasses, as well as combined species frequencies. Carcass swab samples were analyzed across region of collection as well. Chi-square comparisons were made between sampling time-points as well.

Small-ruminant retail samples were calculated for overall frequency, as well as frequency separated by region of sample collection. Significance for all calculations were detected at $P < 0.05$. Chi-square comparisons were made across regions of retail meat collections.

Results

In this study, a total of 334 goat and 544 lamb carcass samples were evaluated for *Salmonella* prevalence. *Salmonella* prevalence in goats at pre-evisceration was 2.17%, 4.10% at post-evisceration and no positive samples found at post-intervention. In lamb carcasses, *Salmonella* prevalence was found to be 3.55% at pre-evisceration, 5.20% at post-evisceration and 3.45% at post-intervention. The frequency of *Salmonella* detected

from goat and lamb carcasses can be seen in Table 11. No significance across sampling time-points was detected for either goat or lamb carcasses ($P = 0.084$ and $P = 0.644$, respectively). Region of sampling had a significant impact on *Salmonella* frequency detected on carcasses (Table 12). *Salmonella* found on small-ruminant carcass surfaces was highest from carcasses harvested in Texas, with no *Salmonella* detected on the carcasses sampled from California ($P = 0.012$). Sampling of carcass surfaces (Table 13) varied significantly by month of collection ($P = 0.0005$).

Distribution of species of retail samples found to be positive for *Salmonella* from total quantity evaluated can be found in Table 14. Retail samples of goat and lamb meat had a *Salmonella* presence detected at 16.82% ($N = 106$). No retail samples tested in Texas were found positive for *Salmonella,* with all positive sample found in either California or the Bahamas ($P = 0.560$). Retail samples collected and frequency of *Salmonella* identified by region of collection can be found in Table 15.

Table 11. Presence of *Salmonella* on sheep and goat carcasses at pre-evisceration, post-evisceration and post-intervention

Time-Point	Specie	# positive	Total Samples	Presence
Pre-evisceration				
	Goat	2	92	2.17%
	Lamb	7	197	3.55%
	Total	9	289	3.11%
Post-evisceration				
	Goat	5	122	4.10%
	Lamb	9	173	5.20%
	Total	14	295	4.75%
Post-intervention				
	Goat	0	120	0.00%
	Lamb	6	174	3.45%
	Total	6	294	2.04%

Table 12. Presence of *Salmonella* on the carcass surface of sheep and goats in the California, Texas and New Mexico

Location	Species	Pre-evisceration		Post-evisceration		Post-intervention	
California							
	Lambs	0/14		0/14		0/14	
	Goats	0/48		0/48		0/48	
	Total	0/62	0.0%	0/62	0.0%	0/62	0.0%
New Mexico							
	Lambs	0/45		2/45		2/45	
	Goats	0		0		0	
	Total	0/45	0.0%	2/45	4.44%	2/45	4.44%
Texas							
	Lambs	7/138		7/114		4/115	
	Goats	2/44		5/74		0/72	
	Total	9/182	4.95%	12/188	6.38%	4/187	2.14%
All states	Total	9/289	3.11%	14/295	4.75%	6/294	2.04%

Table 13. *Salmonella* on sheep and goat carcass surfaces at three time-points (pre-evisceration, post-evisceration and post-intervention) listed by month of collection

Month	Pre-evisceration		Post-evisceration		Post-intervention	
	#Pos/Total	%	#Pos/Total	%	#Pos/Total	%
January	2/5	40.00%	1/5	20.00%	0/5	0.0%
February	2/46	4.35%	1/46	2.17%	0/46	0.0%
March	0/71	0.0%	1/71	1.41%	1/71	1.41%
April	2/76	2.63%	2/76	2.63%	2/76	2.63%
July	0/0	-	1/6	16.67%	0/6	0.0%
September	2/12	16.67	1/12	8.33%	1/12	8.33%
October	0/29	0.0%	4/29	13.79%	0/28	0.0%
November	1/50	2.00%	3/50	6.00%	2/50	4.00%
Total	9/289	3.11%	14/295	4.75%	6/294	2.04%

Table 14. Source of retail samples analyzed for *Salmonella* detailing location of collection and species represented for all samples

Location	Species	# Positive	Total Samples
Bahamas	Goat	0	0
	Lamb	7	24
	Mutton[a]	1	18
	Total	8	42
California	Goat	10	59
	Lamb	0	0
	Total	10	59
Texas	Goat	0	2
	Lamb	0	3
	Total	0	5

[a]In the Bahamas samples labeled as mutton are understood to be derived from a small-ruminant older than 1 year of age, typically with horns. This means the specie could be either goat or sheep, but definitive specie is unknown.

Table 15. Presence of *Salmonella* in small-ruminant retail samples from the Bahamas and U.S. detailed by location of collection

Location	# Positive	Total Samples	Presence of *Salmonella*
Bahamas	8	42	19.05%
California	10	60	16.67%
Texas	0	5	0.00%
Total	18	107	16.82%

Discussion

Salmonella **on carcass surfaces**

Salmonella presence was detected at 2.17% and 3.55% for pre-evisceration goat and lamb carcass surfaces, respectively. This is slightly lower than detection at pre-evisceration, 4.3%, previously detected on lamb carcasses from commercial establishments in the U.S. (Kalchayanand et al., 2007). The increase in *Salmonella* found in both lambs and goats at pre-evisceration, from 3.11% to 4.75% at post-evisceration, indicates potentially unhygienic dressing procedures or contamination in the abattoir facility during removal of the viscera.

The plants sampled in this study used lactic acid, hot water, acetic acid or a combination of lactic acid and 180°F water as the intervention measures prior to chilling the carcass. Studies have shown the use of organic acids as an intervention prior to chilling can minimize the *Salmonella* population growth on carcasses (Cutter & Siragusa, 1994; Dorsa et al., 1998). The numerical reduction found at post-intervention, indicates intervention methods are likely effective in reducing *Salmonella* contamination on the final carcass. A similar study which evaluated lambs from commercial establishments in the U.S. found *Salmonella* to be present on 1.8% of carcasses at post-intervention (Kalchayanand et al., 2007). A study of chilled lamb carcasses in Australia found *Salmonella* prevalence at 0.1% (Phillips et al., 2001). While numerically lower frequency was seen in our study at post-evisceration, an average presence of *Salmonella* on 2.04% of carcasses surfaces at post-intervention indicates potential for more work to be done in order to minimize this frequency.

The sampling locations on a beef carcass with the highest risk of contamination during processing are the foreshank, hindshank and inside round (Rekow et al., 2010). Therefore, the protocols for this study were designed so that the four areas of the carcass swabbed (leg, midline, foreshank, breast/neck) included these three pre-determined areas, making it more likely that the results be an accurate depiction of pathogen presence.

None of the carcasses, goat or lamb, sampled in California were found to have *Salmonella* present at any time-point. The majority of *Salmonella* from carcasses was picked up in Texas, (4.95%, 6.38% and 2.14% for pre-evisceration, post-evisceration and post-intervention). However, in Texas 188 carcasses were sampled, representing the largest proportion of the total carcasses sampled in this study. There were two facilities sampled in Texas, both considered small sized establishments. The dressing procedures or environment of these facilities could have had an influence on contamination of these carcasses. Both Texas facilities process goats, lambs, hogs and cattle in the same facility, as opposed to the facilities in California and New Mexico which process predominantly lambs and goats. Additionally, the carcasses sampled in Texas spanned over a 14-month period, while the sampling in California and New Mexico each represented a 3-day sampling set at one time-point during the calendar year (California in April, New Mexico in November). In order to further investigate the cause of this variation from state to state, additional samples could be analyzed from facilities in California and New Mexico representing different seasons. This could help create a larger sample size to represent these other states, as well as balance the sampling across calendar time-points.

Retail sample *Salmonella* presence

Retail samples represented a known 61 goat meat samples, 27 lamb meat samples and 18 samples ambiguously labeled as mutton. Mutton from the Bahamas, as discussed above, is understood to refer to small-ruminants older than one year, typically with horns. Mutton samples are either from either sheep or goat origin, but exact specie is unknown. Therefore, all retail samples were analyzed together without distinguishing by specie.

The retail presence of 16.82% *Salmonella* detected in this study is concerning, higher than our study found on carcass surfaces of sheep and goats in the U.S. and also higher than previously reported. As discussed above, *Salmonella* was only detected on 2.04% of goat and lamb carcasses harvested in the U.S., much lower than the retail frequency of *Salmonella* detected here. A study from goat available in retail markets of Nepal found *Salmonella* prevalence to be 3.3% (Maharjan et al., 2006). In New Zealand retail lamb and mutton was found to have *Salmonella* prevalence of 1.3% (Wong et al., 2007). The same methods were used for enrichment, screening and confirming presumptive positive samples from the GeneDisc® for retail samples and carcass swab samples, so a difference of methods should not have caused this difference.

Retail meat microbial quality can be influenced by a number of factors including: initial load, pH, water activity, oxygen availability, storage and temperature (Mills & Brightwell, 2014). Arguably most important is the initial concentration and type of bacteria present in the retail package. *Salmonella* are adaptable and can survive in a multitude of diverse mediums. They have been shown to be able to use a wide range of organic substrates for energy, and have a viable pH range of 4.5 to 9.5 (Montville et al.,

2012). Dehydration and cold-storage have been shown to reduce *Salmonella* viability, but the organism may not be completely eliminated (Gruzdez et al., 2012). Packaging type was not tracked in the current study, however the majority of meat samples were either purchased in overwrap packages or as cut pieces bagged at the meat department in the store. Overwrap packaging, while traditional in the retail setting, has been shown to have higher counts of aerobic plate counts and *Salmonella,* as well as quicker spoilage than other packaging types using a vacuum or modified atmosphere (Brooks et al., 2008). The *Salmonella* found in these retail samples could have been a contamination that occurred in the retail setting from equipment, employees or related routes of cross-contamination. Duffy et al. (2001b) found *Salmonella* in pork to be present in 9.6% of retail samples, versus 5.8% of plant acquired samples, with this difference attributed to a contamination in the retail setting. Further work should be done to attempt to identify the source of retail contamination for small-ruminant meat, and effectively reduce this prevalence. While none of the recovered organisms were tested for the presence of virulence factors, the high prevalence detected has potential to be a health risk. Further identification of isolates found could indicate the direct food safety risk, as well as possible suggestion for the identification of the source of this *Salmonella.*

Conclusion

Carcass swabs within this study at pre-evisceration were initially lower than previously reported. Post-evisceration carcass surfaces had the most *Salmonella* present. This likely indicates some sort of contamination during the evisceration and dressing

process. While the prevalence of *Salmonella* decreased after intervention, it was still found more frequently in the current study than previously reported. This again indicates the importance of hygienic dressing procedures and minimizing cross-contamination during processing. Further work should look to find the source of *Salmonella* and methods of reducing it in the end product. Most concerning from these results was the prevalence of *Salmonella* detected in retail samples of small-ruminants. The results in this study were higher than previously reported prevalence for *Salmonella* in retail small-ruminant samples. Future studies should investigate the source of this pathogen in the retail meat and determine its virulence to further assess the food safety risk. These studies could focus on routes of potential contamination after the intervention is applied in the abattoir, prior to packaging, and during presentation in the retail setting.

Chapter 5: *Salmonella* presence in lymph nodes from sheep and goats in the U.S.

Introduction

In the U.S., *Salmonella* are estimated to affect 1.0 million people, causing 11% of total foodborne illness, 30% of foodborne hospitalizations and 28% of deaths from pathogens acquired from food each year (Scallan et al., 2011). Globally *Salmonella* ingested through food can be attributed to approximately 80.3 million illnesses and 155,000 deaths (Majowicz et al., 2010). While the known diversity of food products that have been found to maintain and support the survival of *Salmonella* have broadened, the natural and ubiquitous presence of this organism make it a continuing challenge in food production. *Salmonella* is naturally found in the environment and intestinal tracts of humans, poultry and many livestock species, making it an understood cause of foodborne gastroenteritis when ingested by humans (Jay et al., 2005; Montville et al., 2012; Tortora et al., 2010).

The ability to thrive in a variety of hosts makes *Salmonella* a critical concern with regard to the preparation and processing of animal derived proteins such as meat, milk and eggs. Poultry, cattle, pigs and goats can harbor and shed strains of *Salmonella* without outwardly expressing signs of disease; ultimately leading to illness in humans that have consumed contaminated products (Newell et al., 2010).

In order to assess the risk of *Salmonella* in livestock, prevalence studies are conducted using the carcass surface, hide, lymph nodes, fecal samples and various tissue. Understanding which pathogens and organisms are commonly found within the

gastrointestinal system or in fecal samples from each specie is important because, while those systems are removed from the carcass, there is the potential for intestinal contents to come in contact with and therefore contaminate the meat. The lymphatic system, also plays an important role within the living animal, and cannot entirely be removed from the carcass and resulting meat products. Translocation, the movement of substances, including microorganisms, from the gastrointestinal tract to other nearby organs, such as lymph nodes, has been studied in a variety of animal models (Górski et al., 2006; Steffen & Berg, 1983; Wolochow et al., 1966).

Several studies have reported the prevalence of *Salmonella* found in lymph nodes as a potential source of *Salmonella* contamination in ground meat, especially beef. *Salmonella* prevalence in beef has been shown to be as high as 91% in mesenteric and 55.9% in mandibular lymph nodes (Gragg et al., 2013). Cattle have also been shown to contain *Salmonella* in 1.05% to 76.5% of subiliac lymph nodes (Arthur et al., 2008; Gragg et al., 2013). Mesenteric lymph nodes, the lymph nodes attached to the gastrointestinal tract, are often used as an indicator of overall organism prevalence from the animal. Mesenteric lymph nodes, however, are usually removed as part of the viscera, and therefore are not a direct concern for contamination of meat from the carcass (Arthur et al., 2008). Lymph nodes housed within the fatty tissue of the carcass can easily be included as part of the lean and fat trimmings used to make ground meat (Koohmaraie et al., 2012). Therefore, peripheral lymph nodes, such as the subiliac, can be considered a more reliable indicator of pathogen contamination risk entering the food supply (Gragg et al., 2013).

Current research has shown *Salmonella* prevalence to range from 3% - 17% in a variety of small-ruminant lymph node samples (Chandra et al., 2006; El Tom et al., 1999; Kumar et al., 1973; Samuel et al., 1981; Woldemariam et al., 2005). While research exists about *Salmonella* prevalence found in the lymph nodes of cattle, studies focused on small-ruminant prevalence, especially in the U.S., are limited. The objective of this study was to evaluate the prevalence of *Salmonella* found in mandibular, mesenteric and subiliac lymph nodes collected from ovine and caprine species within the U.S.

Materials and methods

Sample collection

Mandibular, mesenteric and subiliac lymph nodes were collected from sheep and goats harvested at small (1-30 animals per day) and large (800-1,000 animals per day) sized abattoirs located in in Texas, New Mexico and California over a 14-month time period. Each plant used for sample collection was represented by a minimum of three days to avoid any abnormalities from a single day or single group of animals being processed. Mandibular lymph nodes were removed post-exsanguination, after the head was severed. The mandibular nodes are located near the jowls, or back of the jaw closest to the neck. Depending on the method of head removal, these nodes were either attached to tissue along the jowl, or attached to tissue in the neck, at the site of head removal. Mesenteric lymph nodes were removed post-evisceration, targeting a lymph node located closest to the cecum in the viscera. Subiliac lymph nodes were collected from the pocket of fat and tissue near the flank, after evisceration but prior to carcass interventions or

chilling. Subiliac lymph nodes were cut off the carcass, with care being taken to not cut

the lymph node during the removal from the carcass. Whenever possible all three

representative lymph nodes were collected from the same animal. All samples collected

were put into sterile, labeled and separate bags, kept cold ($\leq 4°C$) in clean plastic coolers

with pre-frozen ice packs until able to be transported to the lab. Samples within the

coolers were then transported to Texas Tech University in Lubbock, Texas for immediate

processing.

Lymph node sample processing and *Salmonella* detection

      Processing of the small-ruminant lymph nodes was done by adapting laboratory

methods previously shown to be successful in culturing of *Salmonella* from cattle nodes

(Brichta-Harhay et al., 2011; Gragg et al., 2013). Once in the lab, lymph nodes were

aseptically trimmed of excess fat and tissue with care being taken to avoid cutting of the

node in the laboratory. Tools for trimming nodes were sterilized between each new

sample. A data sheet accompanied each lymph node with notes about the condition of the

lymph node received, record of any cuts made within the trimming process and weight.

Trimmed lymph nodes were weighed, subjected to brief surface treatment to kill

vegetative cells in boiling water, placed in filtered whirlpak™ bags (Nasco; Fort

Atkinson Wisconsin), pulverized with a rubber mallet, enriched in 40 mL of Tryptic Soy

Broth (TSB; Becton, Dickinson and Company; Franklin Lakes, New Jersey), and

homogenized using an automated stomacher (Steward Laboratory Systems; Davie, FL)

for two minutes at 230 RPM. Enrichments were incubated two hours at 25°C, then 12

hours at 42°C. Samples were then processed through Immunomagnetic Separation (IMS; BeadRetriever™ System; Dynal Biotech; Wirral, UK) using Anti-*Salmonella* Dynabeads® (Invitrogen; Grand Island, New York). IMS bead product was transferred to three mL of Rappaport-Vassiliadis *Salmonella* enrichment broth (RV; Oxoid, Hampshire UK), incubated 18-20 hours at 42°C, and then streaked to Xylose Lysine Deoxycholate (XLD; Hardy-Diagnostics, Santa Maria, CA) and brilliant green sulfa (BGS; Remel, Lenexa, KS) agar plates. BGS and XLD plates were incubated 18-20 hours at 37°C.

BGS plates were evaluated for pink or opaque colonies surrounded by a red zone, indication of presumptive positive *Salmonella* as indicated by the FSIS MLG (FSI, 2014). XLD plates were evaluated for red or pink colonies with a black center, as described by the media manufacturer (Hardy Diagnostics). Presumptive positive *Salmonella* colonies from BGS and XLD plates were subjected to latex agglutination (Oxoid; Hampshire, UK) for confirmation using manufacturer's instructions. Isolates of colonies which were agglutination positive were selected for further enrichment into TSB for 24 hours at 37°C, and frozen in duplicate with 20% glycerol for further analysis at a later time.

Statistical analysis

Prevalence of *Salmonella* was calculated using the frequency procedure of SAS (SAS Institute Inc., Cary, NC). Frequency was calculated for each respective lymph node controlling for specie (lamb or goat) as well as frequency with species combined. Frequencies were also calculated for region of sample origin and month of collection.

Significance was detected at $P \leq 0.05$. Chi-square comparisons were made between lymph node types (mandibular, mesenteric and subiliac).

Results

From goats (*N*=311) and lambs (*N*=357), a total of 668 lymph nodes were collected and analyzed from California, New Mexico and Texas (Table 16.). *Salmonella* was found in 1.94% in goat mandibular lymph nodes (*n*=103), 3.85% in goat mesenteric lymph nodes (*n*=104) and 9.62% in goat subiliac lymph nodes (*n*=104). For goats, *Salmonella* frequency was significant between lymph node types (*P* = 0.034).

For lambs, 4.20% of mandibular lymph nodes (*n*=119), 7.56% of mesenteric lymph nodes (*n*=119) and 5.88% of subiliac lymph nodes (*n*=119) were positive for *Salmonella.* No significance was detected among lymph node types from lambs (*P* = 0.599).

Combined lamb and goat prevalence was not significant between lymph node types (*P* = 0.116). *Salmonella* was detected in 3.15% of mandibular lymph nodes (n=222), 5.83% of mesenteric lymph nodes (n=223) and 7.62% of subiliac lymph nodes (n=223). *Salmonella* presence separated by specie for mandibular, mesenteric and subiliac lymph nodes can be seen in Table 17.

Region of animal source was significant in this study (*P* < 0.0001), however, the number of animals represented in Texas is numerically greater than then quantity of animals collected from in California and New Mexico (Table 18.). A representation of lymph node sampling done across calendar time-points can be seen in

Table 19. The month of sample collection was significant ($P < 0.001$), with the highest percent of positive lymph nodes harvested in March.

Of the 225 total animals lymph nodes were collected from, 30 animals had one or more positive lymph nodes (Figure 2).

Table 16. Lymph node sources detailed by state (California, New Mexico or Texas) of sample collection, species and quantity of each type of node analyzed (mandibular, mesenteric and subiliac)

	Species	Mandibular	Mesenteric	Subiliac
California				
	Goats	46	46	46
	Lambs	15	15	15
	Total	61	61	61
New Mexico				
	Goats	0	0	0
	Lambs	45	45	45
	Total	45	45	45
Texas				
	Goats	57	58	58
	Lambs	59	59	59
	Total	116	117	117
All States	Total	222	223	223

Table 17. Total number of samples and frequency found positive for *Salmonella* separated by specie and combined frequencies detected for mandibular, mesenteric and subiliac lymph nodes from small-ruminants in the U.S.

Species	MAN # Pos/ Total	%	MES # Pos/ Total	%	SUB # Pos/ Total	%	% across all nodes within species	*P* - value
Goats	2/103	1.94%	4/104	3.85%	10/104	9.62%	5.14%	*P* = 0.034
Lambs	5/119	4.20%	9/119	7.56%	7/119	5.88%	5.88%	*P* = 0.545
Total	7/222	3.15%	13/223	5.83%	17/223	7.62%	5.54%	*P* = 0.678

Table 18. Presence of *Salmonella* in mandibular, mesenteric and subiliac small-ruminant lymph nodes shown by region of collection, with total samples and frequencies

State	Mandibular		Mesenteric		Subiliac	
	# Pos/Total	%	# Pos/Total	%	# Pos/Total	%
California	0/61	-	0/61	-	0/61	-
New Mexico	0/45	-	0/45	-	0/45	-
Texas	7/109	6.03%	13/117	11.11%	17/117	14.53%

Table 19. Presence of *Salmonella* detected in small-ruminant lymph nodes shown by month of collection

	Mandibular		Mesenteric		Subiliac		Overall lymph node % across month ($P < 0.001$)
	#Pos/Total	%	# Pos/Total	%	# Pos/Total	%	
February	1/24	4.17%	0/24	-	0/24	-	1.39%
March	6/60	10.00%	13/61	21.31%	15/61	24.59%	18.68%
April	0/75	-	0/75	-	0/75	-	0.0%
November	0/45	-	0/45	-	0/45	-	0.0%
October	0/18	-	0/18	-	2/18	11.11%	3.70%

Month and year of collection	Species	Animal ID	Lymph node type MAN	MES	SUB
Feb 2014	G	1	■		
Oct 2014	G	2			■
Oct 2014	L	3			■
March 2015	L	4		■	
March 2015	L	5			■
March 2015	L	6			■
March 2015	L	7			■
March 2015	L	8			■
March 2015	L	9		■	
March 2015	L	10			■
March 2015	L	11	■		■
March 2015	L	12	■		
March 2015	G	13	■	■	■
March 2015	G	14			■
March 2015	G	15		■	■
March 2015	G	16		■	
March 2015	G	17			■
March 2015	G	18			■
March 2015	G	19		■	
March 2015	G	20			■
March 2015	G	21			■
March 2015	L	22		■	
March 2015	L	23	■		
March 2015	L	24		■	
March 2015	L	25		■	
March 2015	L	26		■	
March 2015	L	27	■	■	
March 2015	L	28	■		
March 2015	L	29		■	
March 2015	L	30		■	

Figure 2. Representation of all *Salmonella* positive lymph nodes in this study. A red box indicates a positive node. Multiple red boxes in the same row indicated multiple positive nodes from the same animal.

77

Discussion

  Salmonella was detected in all three lymph node types in samples from both sheep and goats in this study. These data are consistent with previous studies, which reported that *Salmonella* prevalence in small-ruminants to range from 3% - 17% (Chandra et al., 2006; El Tom et al., 1999; Kumar et al., 1973; Molla, 2006; Nabbut & Al-Nakhli, 1982; Samuel et al., 1981; Woldemariam et al., 2005). The three different lymph node types evaluated in this study are important to understanding the potential role of *Salmonella* in small-ruminant carcasses.

  Mandibular lymph node prevalence was numerically lower than subiliac or mesenteric prevalence, but still detectable and therefore an important concern to be aware of. Mandibular lymph nodes in swine have been reported at 12.9% (Vieira-Pinto et al., 2005). In cattle up to 55.9% of mandibular lymph nodes have been shown to have *Salmonella* (Gragg et al., 2013). Since mandibular lymph nodes are generally understood to filter from the salivary glands, mouth, nose and eyes, they could be a reflection of oral exposure to *Salmonella* from environmental causes or other animals (Healthline, 2015).

  Mesenteric lymph nodes are the most frequently studied node for pathogen prevalence in small-ruminants (El Tom et al., 1999; Kumar et al., 1973; Molla et al., 2006; Woldemariam et al., 2005). The overall mesenteric prevalence of *Salmonella* at 5.83% was consistent with the previous data.

  It was once thought that all pathogens from livestock could be contained and therefore removed with the viscera and hide. However research has shown the lymph nodes have the potential to harbor pathogens such as *Salmonella* (Arthur et al., 2008;

Gragg et al., 2013; Samuel et al., 1981). The lymphatic system is designed to transport fluids, immune cells and soluble molecules through lymph nodes, this also includes foreign substances, inflammation associated components, and anything else also within the stream (Gashev & Chatterjee, 2013). Therefore even with hygienic dressing procedures and the proper use of interventions, *Salmonella* contamination of the final product has the potential to occur through lymph nodes.

Subiliac lymph nodes, the nodes most likely to be incorporated into trim to be ground, or sold with the final product, were found to have the highest frequency of *Salmonella* in this study (7.62%). In cattle, *Salmonella* from subiliac lymph nodes has been reported as high as 88% (Gragg et al., 2013; Haneklaus et al., 2012).

Lymph nodes from each respective animal were identified accordingly during sampling and labeling. While the objective of this study was not to determine patterns of *Salmonella* within lymph nodes of the same animal, some animals did show multiple positive lymph nodes. One animal was positive for *Salmonella* in all three lymph nodes (mandibular, mesenteric and subiliac), four animals had two positive nodes, and 25 animals had one lymph node positive for *Salmonella.* A study of lymph nodes from cattle and sheep found that just over 70% of animals with at least one positive lymph node, had more than one serotype of *Salmonella* contained within their node(s) evaluated (Samuel et al., 1981). Vieira-Pinto, M. et al. (2005), however, found that in pigs with multiple positive lymph node, the same serotype of *Salmonella* was always detected in the lymph nodes from the same animal. Serotyping the isolates collected from positive lymph nodes

in our study could give better indication if multiple nodes from the same animal contained the same or different serotypes.

Lymph node mapping, the study of determining routes of lymphatic drainage within the body, is an important studied area within human medicine to better study cancer patterns. While lymph nodes generally drain from the organs and systems local to them, studies of movement through the drainage system have demonstrated complexity and therefore inconsistencies, even between nodes located near each other (Soltesz et al., 2006). Mandibular lymph nodes generally filter from salivary glands, the mouth, nose and eyes (Healthline, 2015). A challenge study which orally exposed swine to *Salmonella* found organism prevalence to be highest in tonsils (93.5%), with prevalence of the same strain also in 54.8% of mandibular lymph nodes (Wood et al., 1989). Cattle challenged subcutaneously with *Salmonella* inoculations acquired infection within the peripheral lymph nodes of the same area, but concentration levels within the nodes were inconsistent (Edrington et al., 2013).

The use of the IMS procedure has been shown to allow detection of *Salmonella* with as low as 1-5 cells/25 g of sample (Cudjoe et al., 1997). Previously found studies that evaluated lymph nodes of small-ruminants did not use IMS methods, but instead relied on selective enrichment and culturing. The use of IMS in this study helped target *Salmonella*, even if it was only present at a low concentration. A study of fed and cull cattle found concentrations of quantifiable subiliac nodes to range from 0.1 to 3.8 $\log_{10}$ CFU/g (Gragg et al., 2013). Further work should be done to quantify the prevalence of *Salmonella* found within the respective small-ruminant lymph nodes. In cattle, direct fed

microbial have been shown to reduce *Salmonella* prevalence in peripheral lymph nodes, indicating the potential to use feeding as a method to reduce *Salmonella* presence in the lymphatic system of ruminants (Vipham et al., 2015).

       Salmonella prevalence in small-ruminant fecal samples has been shown to range from less than one percent to 46.3%, confirming the presence of *Salmonella* in these species (Davies et al., 2003; Duffy et al., 2009; Woldemariam et al., 2005). Our study also found *Salmonella* to be present in mandibular, mesenteric and subiliac lymph nodes in sheep and goats, confirming the presence of this organism in the lymphatic system. Further work should be done to investigate the mode of infection of lymph nodes with *Salmonella* in sheep and goats.

Conclusion

       This study demonstrated *Salmonella* is indeed present in mandibular (3.15%), mesenteric (5.83%) and subiliac (7.62%) lymph nodes of sheep and goats. While this study included samples over a 14-month period, further work should be done to investigate the role season, geography or feed conditions could have on this prevalence. Some animals in our study had multiple lymph nodes positive for *Salmonella*, indicating the potential for further work that could be done to determine patterns in positive nodes from the same animal.

Chapter 6: Summary and Conclusions

The three major foodborne bacterial targets that have had, and continue to have, the spotlight for research efforts, surveillance and discussion in the food industry relative to products derived from livestock are *Salmonella* spp., *Campylobacter* spp. and *E. coli* (Newell et al., 2010). The findings of this study confirm that *Salmonella, Campylobacter,* and pathogenic *E. coli* O157 were all detected in samples from small-ruminants.

Campylobacter prevalence in the fecal samples was the most alarming (80.68%), and could be concerning if these species detected are pathogenic. Detection for this organism was not done on hide, carcass or retail samples, but with a prevalence that high in fecals there is certainly potential for contamination of the carcass. *Salmonella* in small-ruminant fecals was detected at 13.91% and 17.11% on hides. *E. coli* O157 presence in small-ruminant fecals was 15.30% and only 1.50% on hide surfaces. The relationship between hide and fecal presence of *E. coli* O157 deserves further investigation. As discussed earlier, the lower quantity of *E. coli* O157 positive samples detected from the hide could be influenced by fiber length and type.

Lamb and goat carcasses did show *Salmonella* presence throughout the harvest process, although presence was lowest at post-intervention application. This reinforces the need for hygienic dressing procedures and minimizing potential for contamination during harvest. The prevalence of *Salmonella* detected in retail samples, however, was higher than carcass surface presence, and higher than expected (16.82%). Further work could be done in this area to attempt to identify the source of this *Salmonella* in retail

samples and reduce it. Areas of particular interest could be focused on routes of contamination after intervention application, or contamination in the retail setting.

The analysis of lymph nodes did confirm *Salmonella* to be present in mandibular (3.15%), mesenteric (5.83%) and subiliac (7.62%) nodes. Some animals had multiple lymph nodes found to be positive for *Salmonella,* although no serotyping was done in this study to discover if the same organism was present in multiple nodes. While this study included samples over a 14-month period, further work should be done to investigate the effect of season and geography on *Salmonella* presence in small-ruminants.

The presence of these three potentially pathogenic organisms is important to understand for producers, processors and consumers of goat and lamb products. This information from the present study can be especially valuable in trying to better understand and reduce the potential risks presented by these species, in developing countries and globally, as the popularity of small-ruminants as a meat source grows.

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Appendix A:

Carcass Locations for Sampling

1. Leg

2. Midline/Flank area

3. Foreshank

4. Neck
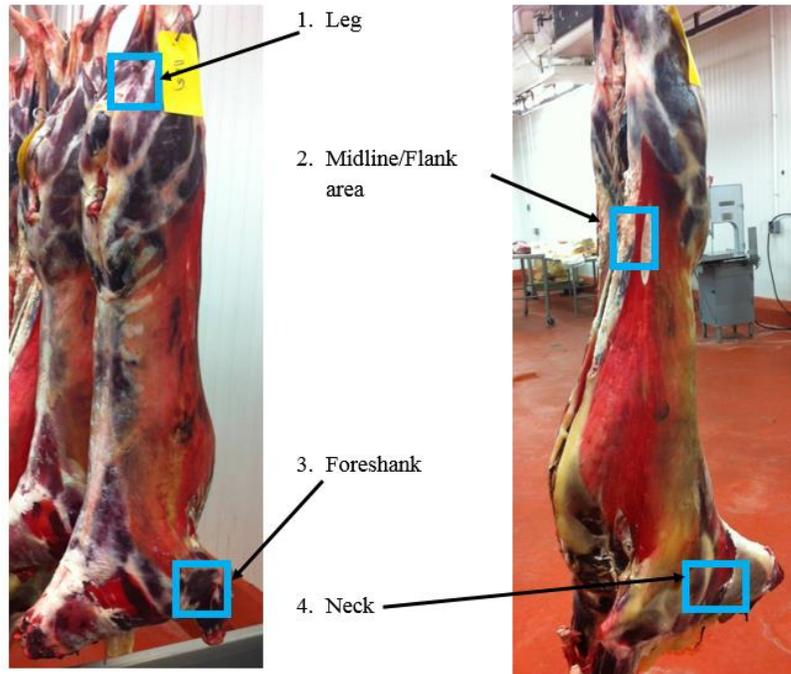
Appendix B:

Chapter 3 SAS Input Code for Data Analysis

```
data chp1sal;
input species$ id$ sal month$ location$ source$;
datalines;

;
run;
title 'Chapter 1 Salmonella KH';
proc freq data=chp1sal;
tables species id sal month location source;
run;
proc freq data=chp1sal;
tables species*id*sal/ chisq measures out=sal;
run;
proc freq data=chp1sal;
tables month*id*sal/ chisq measures out=sal;
run;
proc freq data=chp1sal;
tables location*id*sal/ chisq measures out=sal;
run;
proc freq data=chp1sal;
tables source*id*sal/ chisq measures out=sal;
run;
proc freq data=chp1sal;
tables id*sal/ chisq;
run;
proc freq data=chp1sal;
tables species*sal/chisq;
run;


data chp1salshow;
input species$ id$ sal month$ location$ source$;
datalines;

;
run;
title 'Chapter 1 Salmonella SHOW KH';
proc freq data=chp1salshow;
tables species id sal month location;
run;
proc freq data=chp1salshow;
tables species*id*sal/ chisq measures out=sal;
run;
proc freq data=chp1salshow;
tables month*id*sal/ chisq measures out=sal;
run;
proc freq data=chp1salshow;
tables id*sal/ chisq;
```

```
run;
proc freq data=chp1salshow;
tables species*sal/chisq;
run;

E.COLI RUN

data chp1ec;
input species$ id$ ec month$ source$ location$;
datalines;


;
run;
title 'Chapter 1 E.coli  KH';
proc freq data=chp1ec;
tables species id ec month source location;
run;
proc freq data=chp1ec;
tables species*id*ec/ chisq measures out=ec;
run;
proc freq data=chp1ec;
tables month*id*ec/ chisq measures out=ec;
run;
proc freq data=chp1ec;
tables location*id*ec/ chisq measures out=ec;
run;
proc freq data=chp1ec;
tables source*id*ec/ chisq measures out=ec;
run;
proc freq data=chp1ec;
tables id*ec/ chisq;
run;
proc freq data=chp1ec;
tables species*ec/chisq;
run;

data chp1ecshow;
input species$ id$ ec month$ source$ location$;
datalines;

;
run;
title 'Chapter 1 E.coli Fecal SHOW KH';
proc freq data=chp1ecshow;
tables id species ec month source location;
run;
proc freq data=chp1ecshow;
tables species*id*ec/ chisq measures out=sal;
run;
proc freq data=chp1ecshow;
tables month*id*ec/ chisq measures out=sal;
run;
```

```
proc freq data=chp1ecshow;
tables id*ec/ chisq;
run;
proc freq data=chp1ecshow;
tables species*ec/chisq;
run;


CAMPY RUN

data chp1campy;
input species$ id$ campy month$ location$ source$;
datalines;

;
run;
title "Chapter 1 Campylobacter KH";
proc freq data=chp1campy;
tables species id campy month location source;
run;
proc freq data=chp1campy;
tables species*id*campy/ chisq measures;
run;
proc freq data=chp1campy;
tables month*id*campy/ chisq measures;
run;
proc freq data=chp1campy;
tables location*id*campy/ chisq measures;
run;
proc freq data=chp1campy;
tables source*id*campy/ chisq measures;
run;
proc freq data=chp1campy;
tables id*campy/ chisq;
run;
proc freq data=chp1campy;
tables species*campy/chisq;
run;
```

Appendix C:

Chapter 4 SAS Code for Data Analysis

```
data chp2retail;
input species$ id$ sal month$ location$;
datalines;


;
run;
Title 'Chapter 2 Retail KH';
proc freq data=chp2retail;
tables species id sal month location source;
run;
proc freq data=chp2retail;
tables species*sal/ chisq measures;
run;
proc freq data=chp2retail;
tables month*sal/ chisq measures;
run;
proc freq data=chp2retail;
tables location*sal/ chisq measures;
run;
proc freq data=chp2retail;
tables source*sal/ chisq measures;
run;

CARCASS SURFACE (SALMONELLA) INPUT

data chp2carcass;
input species$ id$ sal month$ location$ source$;
datalines;


;
run;
title "Chapter 4 Sal on Carcass KH";
proc freq data=chp2carcass;
tables species id sal month location source;
run;
proc freq data=chp2carcass;
tables species*id*sal/ chisq measures;
run;
proc freq data=chp2carcass;
tables month*id*sal/ chisq measures;
run;
proc freq data=chp2carcass;
tables location*id*sal/ chisq measures;
run;
proc freq data=chp2carcass;
```

```
tables id*sal/ chisq;
run;
proc freq data=chp2carcass;
tables species*sal/chisq;
run;
proc freq data=chp2carcass;
tables month*sal/chisq;
run;
proc freq data=chp2carcass;
tables location*sal/chisq;
run;
```

Appendix D:

Chapter 5 SAS Code for Data Analysis

```
data chp3ln;
input species$ ln$ sal month$ location$ source$;
datalines;


;
run;
Title 'Chapter 5 Lymph Nodes KH';
proc freq data=chp3ln;
tables species LN sal month location source;
run;
proc freq data=chp3ln;
tables species*ln*sal/ chisq measures;
run;
proc freq data=chp3ln;
tables month*ln*sal/ chisq measures;
run;
proc freq data=chp3ln;
tables location*ln*sal/ chisq measures;
run;
proc freq data=chp3ln;
tables ln*sal/ chisq;
run;
proc freq data=chp3ln;
tables species*sal/chisq;
run;
proc freq data=chp3ln;
tables month*sal/chisq;
run;
proc freq data=chp3ln;
tables location*sal/chisq;
run;
```