# PREHARVEST *ESCHERICHIA COLI* O157:H7 VACCINATION OF BEEF CATTLE: INDUSTRY-WIDE ACCEPTANCE THROUGH A BEEF PRODUCTION LIFECYCLE APPROACH.

by

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B.S., South Dakota State University, 2002
DVM, Iowa State University, 2005

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine/Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2010

# Abstract

*Escherichia coli* O157:H7 is responsible for over 70,000 cases of human illness every year in the United States. Most cases occur in children under the age of five, the elderly, or other immune-compromised people. A small percentage of these cases will develop a life threatening complication, hemolytic uremic syndrome. Cattle are the reservoir host for *E. coli* O157:H7 and serve as the main source of contamination of meat products and other food sources. The beef cattle industry is diverse with producers caring for as few as one to as many as thousands of cattle. The first objective of this research was to examine three major production systems (conventional, organic, and natural) in the U.S. and the published performance effects of the various technologies used in each system. The second objective was to determine if a newly licensed *E. coli* O157:H7 SRP$^{®}$ (SRP) vaccine administered to cows pre-partum could achieve successful passive transfer in their offspring. The third objective was to determine if colostrum obtained from SRP vaccinated heifers could protect against an oral challenge with an *E. coli* K99$^{+}$ strain. The fourth objective was to examine the shedding characteristics, health, and performance effects of calves born to SRP-vaccinated cows that also receive SRP vaccination themselves. The technologies used in conventional beef cattle production resulted in significant improvements in health and performance of beef cattle. Vaccinating cows pre-partum with SRP resulted in passive transfer in calves consuming their colostrum. Calves that achieved successful passive transfer shed less *E. coli* K99$^{+}$ and had improved fecal consistency compared to placebo. When calves were vaccinated with SRP at branding, weaning, and arrival to the feedyard there was no difference in fecal *E. coli* O157:H7 shedding on arrival to the feedyard or at harvest. Vaccinating calves with SRP had no effects on performance or health outcomes. Vaccinating cattle with SRP may provide protection against other pathogenic *E. coli* strains and warrants further investigation. The timing of vaccination appears to be an important consideration in order to ensure maximum vaccine efficacy.

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Approved by:

Major Professor
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This thesis is dedicated to two great men, Dr. Dale R. Hendrickson and Dr. James H. Myers. Dr. Hendrickson allowed me, a wild-eyed 8th grader to begin volunteering at his veterinary clinic in Rapid City, South Dakota. He eventually hired me for what I thought was not enough, but I was never aware how much I was getting from him until veterinary school and my first year of practice. Then I realized that all of those afternoons, evenings and weekends that I spent with him were worth more than I could have ever paid him. He is an outstanding role model of hard work, patience, compassion and brilliance.

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These two men are why I am in veterinary medicine and why I have dedicated my life to serving the beef industry and all the people involved with it. Thank you from the bottom of my heart, this thesis is for the two of you.

# Preface

Chapters two and three in this dissertation entitled: "Analysis of modern technologies commonly used in beef cattle production: Conventional beef production versus nonconventional production using meta-analysis", and "Characterization of specific passive immunity stimulated by vaccination of beef cows grazing native range with *E. coli* O157:H7-SRP®" were submitted for publication in the *Journal of Animal Science* and *The Bovine Practitioner*, respectively. The text and figures within these chapters are formatted according to the guidelines that were specified by the journal.

# CHAPTER 1 - Literature Review

## *Escherichia coli*

Since their discovery in 1885 by Theodor Escherich, a German pediatrician, *Esherichia coli* have become one of the most studied group of bacteria. In addition to study of the organism itself, *Escherichia coli* have been used as models to learn about biochemical reactions and processes that eventually gave rise to modern bacterial taxonomy; the organism is now being used as a carrier for gene libraries and phage research regarding disease treatment (Gyles 1994a). *Escherichia coli* belong to the family *Enterobacteriacae* and are morphologically, biochemically and genetically (DNA) related to the genus *Shigella* bacterium. It has been proposed that the two genera be combined. A reclassification has been delayed due to the adoption of the separate terminologies by medical microbiologists and the resulting confusion that would possibly ensue (Brenner 1984). *Escherichia* is the first genus of the family *Enterobacteriacae* and can be identified morphologically as gram-negative rods that may be motile (due to a peritrichuris flagella) or non-motile (Brenner 1984). All *Escherichia* are classified as facultatively anaerobic due to a dual respiratory and fermentive type of metabolism (Brenner 1984). The colony morphology of *E. coli* can vary from rough to smooth with mucoid and slime-producing types possible. Most strains of *E. coli* also ferment lactose which is used in culturing techniques from biological samples but not all strains of *E. coli* will ferment lactose. Modern microbiological classification techniques rely on the amount of Guanine and Cytosine present in the organism to classify bacteria into genetic trees. For *E. coli,* the mol% G + C of *E. coli* DNA is 48-52 (Orskov 1984).

## *Classification*

With the emergence of a growing number of *E. coli* strains involved in disease outbreaks, early researchers began to classify subtypes of *E. coli* in order to describe differences observed in the course of particular outbreaks. Kauffman (1947) was the first to classify *E. coli* based on serological methods (Lior 1994). Thus was born the process and field of *E. coli*. serotyping that became the standard form of identification in medical microbiology.

Identification of *E. coli* serotypes is based on serological reactions to somatic antigens O, K, H, and F. The 'O' antigens are derived from the outer lipopolysaccharide (LPS) membrane of *E. coli* and are not inactivated by heat (Lior 1994). There are two groups of 'O' antigens: the first group is classified as immobile during electrophoresis and tends to be associated with extra-intestinal disease; the second group is classified as mobile during electrophoresis and tends to be associated with dysentery-like disease (Orskov & Orskov 1972). There are also a number of reports of cross-reactions of 'O' antigens between *Shigella*, *Salmonella* (Orskov and Orskov 1984; Stenutz et al. 2006), *Vibrio cholera,* and *Citrobacter* strains (Winkle et al. 1972).

The 'K' antigen designation was proposed by Kauffmann (1947) to describe the inability of bacterial suspensions to agglutinate until being heated to 100°C. This group has largely been abandoned for the purpose of research due to an inability to produce antibodies in rabbits and lack of clinical usefulness. The remaining 'K' antigen designations are K1 & K5 in human diseases (Lior 1994) and K88 & K99 in animal diseases (Jones & Rutter 1972; Jin & Zhao 2000; Smith & Linggood 1972).

The 'H' antigens are associated with the protein flagellin in the flagella of motile *E. coli* (Lior 1994). Most of the *E. coli* 'H' antigens are specific and show little to no cross-reactivity

(Ewing 1986).  The 'H' and 'O' antigens are regarded as key markers of the pathogenicity of the various strains of *E. coli* of both intestinal and extraintestinal diseases (Lior 1994).

The 'F' antigens represent proteins associated with fimbrae or pilli that are present on the outer surface of *E. coli*.  Fimbrae and pilli are necessary for attachment and colonization of *E. coli* to mucosal surfaces (Beachey 1981).  One strain of *E. coli* can display several fimbrae of differing antigenic types.  The number of 'F' antigens continues to increase despite the difficulty of serotyping it.  Two of the fimbrial types are associated with K88 (F4) and K99 (F5; Lior 1994).

### *Pathogenic Classification*

Another form of classification for *E. coli* strains is related to enteric diseases and was first proposed by Neter et al. (1955).  This classification system combines multiple serogroups with similar pathogenic effects under a single entity called enteropathogenic (EPEC) *E. coli*.  The system has been expanded relatively recently to include six major groups of enteric disease-related *E. coli* that are based on virulence mechanisms of group members (Kaper et al. 2004; Nataro & Kaper 1998):  1) enteropathogenic *E. coli* (EPEC), 2) enterohemmorhagic *E. coli* (EHEC), 3) enterotoxigenic *E. coli* (ETEC), 4) enteroaggregative *E. coli* (EAEC), 5) enteroinvasive *E. coli* (EIEC), and 6) diffusely adherent *E. coli* (DAEC).

Enteropathogenic *E. coli* (EPEC) are one of the most studied and understood group of pathogenic *E. coli* (Kaper et al. 2004).  Most *E. coli* microbiologists agree on the classification criteria for EPEC as production of a characteristic attaching and effacing lesion on histology on small intestinal cells and no production of Shiga, Shiga-like, or verocytotoxins (Kaper 1996).  Even though EPEC do not produce toxins they still cause disease in humans and animals (Spears et al. 2006).

The majority of the virulence mechanisms for EPEC and EHEC are encoded on 'O' islands or plasmids. The recent sequencing of an EPEC strain E2348/69 (Iguchi et al. 2009) and comparisons made to *E. coli* O157:H7 strains (Hayashi et al. 2001 & Perna et al. 2001) revealed that 69 of the 177 'O-specific' islands have >90% homology across EPEC and EHEC strains (Spears et al. 2006); however, 14 of these islands have less than 55% homology between the two groups indicating the potential for frank differences in virulence mechanisms (Spears et al. 2006).

The ability to produce the characteristic attaching and effacing lesion of EPEC is encoded by genes on the pathogenicity island called the *locus of enterocyte effacement* (LEE; McDaniel et al. 1995). The LEE encodes an outer-membrane protein, intimin, which is involved in the intimate attachment of *E. coli* to the enterocyte and also encodes for the type III secretion system, chaperones, and effector proteins responsible for the characteristic pedestal formation between the bacteria and the enterocyte (Kaper et al. 2004).

A subdivision of EPEC bacteria has also been described based on the presence or absence of the EAF (i.e., EPEC adherence factor) plasmid that encodes a type IV pilus called the bundle-forming pilus (BFP). BFP are important for initial binding to host cells, mediating interbacterial adherence, and formation of three-dimensional micro-colonies (Giron et al. 1991 & Cleary et al. 2004). The first EPEC subdivision, termed typical EPEC, contain the EAF plasmid. Conversely, atypical EPEC lack the EAF plasmid. In industrialized countries, the predominant EPEC isolated from human diarrheal cases are atypical; however typical EPEC are the predominate isolates in developing countries (Trabulsi et al. 2002; Chen & Frankel 2005).

Enterohemorrhagic *E. coli* (EHEC) is one of the most intensely studied groups of *E. coli*. Its founding strain is *E. coli* O157:H7. It was identified in 1982 during the investigation of

bloody diarrhea outbreaks in humans in Oregon, Michigan, and Canada (Riley et al. 1983 & Stewart et al. 1983). *Escherichia coli* O157:H7 is also the predominant EHEC responsible for hemorrhagic colitis and hemolytic uremic syndrome (HUS) in the U.S.; other non-O157 EHEC group members are more common in other parts of the world (Naylor et al. 2005).

The defining characteristic of the more than 100 E. coli strains in the EHEC group is production of verotoxin or shiga-like-toxin (Lior 1994). These toxins are encoded on what are believed to be acquired bacteriophages. There are two main variants of these toxins. Shigella-like toxin 1 (Stx1) is nearly identical to the enterotoxin of *Shigella dysentariae* (O'Brien & Holmes 1987; Nakao & Takeda 2000) and Shigella-like toxin 2 (Stx2) is the toxin most often associated with human disease (Boerlin et al. 1999). The difference between Stx1 and Stx2 in the development of human disease may be due to differences in the B-subunit of the toxin (Fraser et al. 2004). The B-subunits determine the cell-binding specificity and allow the translocation of the A-subunit into the cell. Once inside the cell the A-subunit is cleaved to produce a peptide with N-glycosidase activity that inhibits protein synthesis by cleaving of the 28s ribosomal RNA (Spears et al. 2006). Lingwood (1996) and Lingwood et al. (1987) demonstrated that the B-subunit binds to the receptor Gb3/CD77 to initiate translocation of the A-subunit. CD77/Gb3 receptors are present on human endothelial cells and also on kidney glomerular cells but not bovine endothelial cells (Boyd & Lingwood 1989; Adler & Bollu 1998). This difference in initial binding is likely one of the reasons we see hemorrhagic disease in humans and not in the carrier animal, cattle. This class of *E. coli* will be discussed in greater detail in the section specifically on *E. coli* O157:H7 that follows.

Enterotoxigenic *E. coli* (ETEC) first emerged in 1971 in human males in India with acute diarrhea (Gorbach et al. 1971; DuPont et al. 1971); however, the relationship between isolating

*E. coli* that produced enterotoxin and colibacillosis was first identified in calves by Smith and Halls (1967).  The distinguishing characteristic of this group of *E. coli* is the production of enterotoxin and the presence of colonization factor antigens (CFA).  At least 21 distinct CFA have been identified in human derived ETEC, which are distinct from CFA in animal derived ETEC.  ETEC target the small intestine and colonize along the mucosa where they produce one or both of two enterotoxins, heat-labile toxin (LT) and heat-stabile toxin (ST; Elsinghorst 2002).

The initial adherence of the ETEC to the mucosa is facilitated by the CFA binding to the host cell using one or possibly multiple binding sites located along the filamentous structure (Gaastra and Svennerholm 1996).  The colonization factor for binding sites on host enterocytes is host specific.  An example is the host specificity observed in *E. coli* strains expressing K88 fimbriae; they only cause disease in pigs (Jin and Zhao 2000; Francis et al. 1999).  After CFA binding, the organism colonizes on the surface of the mucosa to allow for a localized secretion of enterotoxin (Zafriri et al. 1987).  The host response to the toxin leads to a net excretion of fluid from the enterocyte into the small intestine, especially in the jejunum (Banwell et al.1971).  This net fluid loss results in the most common clinical sign of *E. coli* infection, watery diarrhea.  Forty-six percent of ETEC isolates release ST alone, 25% release LT alone and approximately 29% of isolates express both ST and LT (Wolf 1997).

LT toxins are structurally and functionally similar to the cholera toxin produced by *Vibrio cholera* (Spangler 1992).  Two types of LT have been characterized.  LT-I toxins are present in both human and animal isolates and can be neutralized by cholera toxin antisera.  LT-II toxins are primarily found in animal derived isolates but cannot be neutralized by cholera toxin antisera.  Interestingly, the production of LT-II has not been associated with diarrhea (Elsinghorst 2002).

Similar to LT toxins, there are two types of heat-stable (ST) enterotoxins. In addition to being heat-stable, these toxins are secreted locally into the gastrointestinal medium where they can have a broader effect range (Rasheed et al. 1990; Okamato & Takahara 1990; Kupersztoch et al. 1990). The first type, ST-I toxin is functionally distinct from ST-II. ST-I bind to the extracellular domain of guanylate cyclase C (GC-C) that is found in the brush-border membrane of intestinal epithelial cells (Hasegawa et al. 1999). The ST-I toxin increases extracellular secretion of chloride by binding the GC-C receptor more effectively than guanylin (Caprick & Gariepy 1993). This binding increases intracellular cGMP which stimulates chloride excretion by crypt cells and inhibits sodium and chloride adsorption by villus epithelial cells. The net increase is to increase fluid accumulation in the gut lumen via osmotic pull (Forte et al. 1992). ST-I toxin is homologous to EAST1 toxin in enteroaggregative *E. coli* (Savarino et al. 1991), as well as other heat-stable toxins found in *Yersinia enterocolitica* and *Vibrio cholera* non-O1 strains (Elsinghorst 2002). ST-II toxin has been implicated in inciting histologic damage to the intestinal epithelium and also increasing intestinal motility; however, ST-II is restricted mainly to porcine ETEC strains and only recently has been found to be active in rats and mice if protected from proteolysis via trypsin in the gut (Whipp et al. 1987; Hitotsubashi et al. 1992). ST-II is rarely the only enterotoxin expressed by porcine ETEC strains. The sole role of ST-II toxin in the pathogenic diarrhea process is likely minor. The reason for its prominence in disease causing ETEC strains is more likely due to its genetic code location being on plasmids that also encode LT, ST-I, colonization factors, drug resistance, colicin production and transfer functions (Gyles 1994b).

Enteroaggregative *E. coli* (EAEC) is the pathotype described most recently (Nataro et al. 1987; Vial et al. 1988) of the six major *E. coli* pathotypes. It is characterized by its "stacked

bricks" appearance on HEp-2 cells (Nataro et al. 1987) and lack of secretion of heat-labile or heat-stable enterotoxins (Nataro & Steiner 2002). EAEC has been linked to diarrheal outbreaks in travelers and in HIV patients in both developed and developing nations (Nataro et al. 2006; Ruttler et al. 2002). EAEC is most associated with inducing a persistent, mucoid diarrhea in humans. Interestingly, there is no known pathogenic animal strains (Fang et al. 1995; Nataro et al. 1995). Data from a recent meta-analysis indicated that EAEC is a cause of acute diarrhea in 15% of children in developing countries and in 4% of children in industrialized nations (Huang et al. 2006a). The ability of EAEC to elicit clinical disease is complicated due to the interaction of EAEC virulence heterogeneity and differing host tolerance levels, which result in varying levels of clinical disease (Elias et al. 2002; Nataro et al. 1995; Huang 2006b).

Clinical disease induction by EAEC involves three stages (Huang et al 2006b): stage 1 involves adherence to the intestinal mucosa via aggregative adherence fimbriae (AAF) and adherence factors; stage 2 involves production of mucus by the bacteria and the host cell forming a biofilm over the surface of the enterocyte; stage 3 involves release of toxins eliciting an inflammatory response, mucosal toxicity, and intestinal secretion.

Stage 1 is driven by the three structural subunits of the AAF which are encoded on the 60-65 MDa pAA plasmid. Two of these structural subunits of the AAF are regulated by a transcriptional regulator AggR (Huang 2006b) that also regulates the expression of a low-molecular weight secretory protein termed dispersin (Sheikh et al. 2002). Dispersin is responsible for the dispersal of EAEC across the mucosa which allows for the adherence and aggregation process to begin. This protein has also been found to be highly immunogenic which makes it a potential vaccine candidate (Nataro et al. 1995). Stage 2 of the pathogenesis involves excessive mucus production from both the bacteria and the intestinal mucosa. The formation of

this dense biofilm aids in the survival of EAEC and is the source of the characteristic mucoid diarrhea. Little is known about biofilm production in EAEC but it does appear to be dependent on two genes (Fis & yafK) that are also mediated by AAF (Sheikh et al. 2001). Stage 3 involves the release of toxins with the corresponding inflammatory response, mucosal toxicity and intestinal secretion. The overall, combined action of these toxins is destructive to the tips and sides of intestinal villi and enterocytes (Huang et al. 2006b).

The three most described toxins involved in the EAEC pathogenesis are plasmid-encoded toxin (Pet), EAEC heat-stable enterotoxin (EAST1) and Shigella enterotoxin 1 (ShET1; Huang et al. 2006b). It should be noted that, EAST 1 is not unique to EAEC but can be found also in ETEC, EHEC, EPEC and in DAEC (Savarino et al. 1991). The host immune response to infection with EAEC and subsequent clinical signs can vary depending upon the genetic composition of the individual (Jiang et al. 2003); however, none of the above genes or virulence factors is conserved among all EAEC. Few of these factors are unique to EAEC. This, combined with host-to-host susceptibility variation, results in sporadic and inconsistent estimates of prevalence and pathogenicity (Huang et al. 2006b; Weintraub 2007).

The Enteroinvasive *E. coli* (EIEC) class of organisms shares a number of somatic antigens with shigellae and incites similar diarrheal disease (DuPont et al. 1971). This intimate relationship between shigellea and EIEC has existed for centuries. Infections affect millions with diarrhea/dysentery each year and may be responsible for 170,000 deaths annually (Niyogi 2005; Black 1993). The unique features of this group of *E. coli* are the presence of a large virulence plasmid, their ability to invade epithelial cells, and their ability to then move from cell to cell (Parsot 2005). EIEC is relatively simple to distinguish it from normal-flora *E. coli* because EIEC is non-motile. In addition, 70% of isolates cannot ferment lactose and do not

decarboxylate lysine.  Conversely, they share these features with *Shigella* species.  This, along

with the similar disease presentation in humans, has translated into many researchers and

epidemiologists combining EIEC and *Shigella* into a common disease category called

Shigellosis.  Humans are the reservoir host for EIEC; very few animal hosts have been identified

(Day & Maurelli 2002).

Day and Maurelli (2002) described the four hallmarks of virulence for EIEC: 1) ability to

induce diarrhea, 2) ability to invade epithelial cells of the intestine, 3) ability to multiply inside

the epithelial cells, and 3) ability to spread from cell to cell.  EIEC organisms benefit from the

initial diarrhea in the jejunum because it provides a transport mechanism to the colon.  In order

to cause clinical disease, EIEC must then go on to invade the epithelium of the large intestine

(LaBrec et al. 1964).  Entry to the epithelial cell is initiated by the type III secretory system

analogous to that described for EPEC strains (Parsot 2005).

The third hallmark of virulence is replication once inside the intestinal epithelium.

Strains that have reduced ability to multiply once inside the epithelial cell are unable to elicit

clinical disease (Formal et al. 1965).  Strains that result in clinical disease must arrive in the

colon, invade the epithelial cell, and multiply within the epithelial cell, and then move from cell

to cell while remaining in the confines of the intracellular space.  The fourth hallmark of

virulence was demonstrated by using organisms that had the capacity to enter epithelial cells and

multiply but lacked the ability move from cell to cell.  These organisms failed to induce clinical

disease (Day and Maurelli 2002).  The ability of EIEC strains to invade epithelial cells and move

from cell to cell is encoded on a large (220 kb) virulence plasmid (Parsot 2005).  A unique

quality of EIEC is that it can regulate the expression of virulence in response to temperature.

Strains grown at 37°C are able to invade epithelial cells; however, strains grown at 30°C are

unable to invade.  This control mechanism mimics similar mechanisms of other pathogens such as *Salmonella* Typhimurium, *Yersinia* species and *Listeria monocytogenes* (Small and Falkow 1988; Parsot 2005).

Diffusely adherent *E. coli* (DAEC) are genetically related to EAEC (Czeczulin et al 1999).  DAEC are differentiated from EAEC by a characteristically diffuse pattern of binding to HEp-2 cells in culture (Nataro & Steiner 2002).  There have been no published outbreaks of enteric disease in humans or animals attributable to DAEC, nor have volunteer challenge studies yielded disease induction (Tacket et al. 1990).  In contrast, epidemiological studies have noted an association with diarrhea in both developing and industrialized nations in adults and children but not infants (Baqui et al. 1992; Gunzburg et al. 1993; Jallat et al. 1993; Levine et al. 1993).  The role of DAEC in urinary tract (UTI) and kidney infections in humans is much more established (Servin 2005).  Due to the inability to demonstrate or replicate enteric disease induction with DAEC very little about the pathogenesis is known.  Recently, more work has been completed on the pathogenesis and virulence factors with regard to the DAEC strains involved in human UTI and pyelonephritis.  Genetic and molecular studies proposed that DAEC be placed into two groups.  The first group of DAEC are those strains that posses Afa/Dr adhesions, and the second group are strains that posses the adhesion involved in diffuse adherence (AIDA-I; Servin 2005).

### *Escherichia coli*   O157:H7

*Escherichia coli* O157:H7 is the flagship organism for the EHEC group and can cause a wide range of symptoms from no detectable illness to life threatening illness (Griffin & Tauxe 1991). *Escherichia coli* O157:H7 is the main organism responsible for causing the potentially fatal hemorrhagic colitis and hemolytic uremic syndrome in humans (Riley et al. 1983).  Ruminants, especially cattle, are regarded as the primary reservoir of *E. coli* O157.  Infection

usually occurs through consumption of contaminated food products, contaminated water sources, direct contact with animals, or direct contact with infected persons (Rangel et al. 2005). The infectious dose of *E. coli* O157 required to cause clinical disease is quite low at 50-100 organisms (Tilden et al. 1996). The incubation time from ingestion of the organism to the manifestation of clinical disease ranges from 1-9 days, with an average of 3-5 days (Griffin & Tauxe 1991). In 2009, this organism had an incidence of 0.99 per 100,000 people in the United States with the highest incidence of morbidity occurring in children < 4 years of age (CDC 2010). Relatively low incidence enabled the U.S. to meet its *Healthy People 2010* target of ≤ 1.0 cases per 100,000 people of STEC O157 infection (U.S. Department of Health and Human Services 2000). The greatest percentage of people hospitalized occurred in those aged > 50 years old; 1.5% of those resulted in death (CDC 2010). Each year approximately 73,000 people become infected with *E. coli* O157 (Mead et al. 1999), costing the U.S. economy approximately $1 billion per year (USDA-ERS 2001) in health care and life-related costs. Of the tracked and reported foodborne illnesses, *E. coli* O157 infections have a relatively low incidence compared to those of *Salmonella* (15.19 per 100,000), *Campylobacter* (13.02), *Shigella* (3.99) and *Cryptosporidium* (2.86; CDC 2010). The interest in the organism is likely due to the small percentage of infections that result in the life-threatening complication known as Hemolytic Uremic Syndrome (HUS). In 2008, there were 64 cases of HUS in people under the age of 18 years; two-thirds of these cases occurred in children under the age of 5 years (CDC 2010). There has been a disproportionate amount of research time and money dedicated towards the study of this single serotype and its virulence properties because of this debilitating and even fatal response to infection.

Research efforts over the last 30 years have identified two major virulence factors that contribute significantly to disease induction and an additional area of putative virulence factors that, when combined, contribute to the survivability of *E. coli* O157 in the host. These virulence mechanisms have been the entire subject matter of several books dedicated to in-depth descriptions of each factor and their effects on the survival and pathogenicity elicitation of the host (Donnenberg 2002; Sussman 1997). The two key features of *E. coli* O157 that significantly increase its virulence are shiga toxin production and presence and functionality of the chromosomal pathogenic island termed the *Locus of Enterocyte Effacement* (LEE). The lesser-known putative factors are usually controlled via the plasmid O157 (pO157) which is capable of replicating independently of chromosomal DNA (Lim et al. 2010).

### *Shiga Toxin Production*

The production of shiga toxin (Stx) is the most important virulence factor of *E. coli* O157, as it is considered essential for disease induction in humans. Stx is a bacteriophage-encoded cytotoxin with two main types, Stx1 and Stx2. Strains can posses the genetic material to encode for either Stx1, Stx2 or both. Strains encoding Stx2 are more toxic to humans than strains possessing Stx1 or both Stx1 and Stx2 (Boerlin 1999).

One of the differences between Stx2 & Stx1 may lie in the B-subunit (Fraser 2004). Both Stx1 & Stx2 are $AB_5$ toxins, so called because the B-subunit is responsible for binding to the host-specific cell receptor called Gb3 (Nataro & Kaper 1998). The Gb3/CD77 receptor is present in human endothelial cells and human kidney glomerular cells (Boyd & Lingwood 1989; Alder & Bollu 1998). The amount and type of Gb3 receptor present on the cell surface determines the susceptibility of the cell to Stx (Gyles 2007). After binding, the A subunit is internalized into the cytoplasm via receptor-mediated endocytosis (Sandvig et al. 2004). It is

transported subsequently to the Golgi apparatus and endoplasmic reticulum where it inhibits

protein synthesis occurring at the 28s rRNA of the 60s ribosome and induces apopotosis (Gyles

2007; Lim et al. 2010).  The dissemination of Stx to other than the intestine, and its effects on

them, is not well understood.

### *Locus of Enterocyte Effacement*

Adherence to intestinal cells is associated with highly pathogenic strains of *E. coli* O157

serotypes (Boerlin 1999).  Not all Shigella-like Toxin producing *E. coli* (STEC) produce the

characteristic attaching and effacing (AE) lesion.  STEC that are *eae*-positive (i.e., *E. coli*

attaching and effacing) tend to attach to epithelial cells more strongly and produce the

characteristic AE lesion (Nataro and Kaper 1998).  These *eae*-positive strains are also

responsible for the majority of infections that result in HUS and, thus, have been identified as a

significant risk factor (Ethelberg et al. 2004).  A pathogenicity island on the *eae* gene termed the

locus of enterocyte effacement (LEE) is of particular interest.  It encodes most of the proteins

necessary for the formation of the AE lesion.

The LEE is organized into 5 major operons, LEE1 to LEE5.  Operons LEE1 to LEE3 lead

to the formation of the type III secretory system which is a unique mechanism for transfer of

proteins between the bacterium and the enterocyte to which it attaches.  Operon LEE4 forms the

protein translocation system used to transfer material between the cells.  Operon LEE5 is

responsible for the outer membrane system.  Using intimin and its own receptor (i.e.,

translocated intimin receptor; TIR) the outer membrane system facilitates attachment to the

enterocyte (Gyles 2007).

The type III secretory system is not unique to *E. coli.*  It can be found in many other

bacteria such as *Yersinia*, *Shigella*, *Salmonella* and *Pseudomonas*.  Half of the 25 proteins that

code for the secretory mechanism are conserved in most of the type III systems (Mota & Cornelis 2005). The type III secretion system uses a syringe and injector-type of secretory system that links the cytoplasm of the bacterium to that of the enterocyte. Once the two cells are penetrated, effector proteins are transferred to the enterocyte through the structure. One of these proteins is TIR which binds to enterocyte proteins and is inserted into the membrane of the enterocyte. There it acts as a receptor for intimin from the bacterial surface. This signals a number of cascades that result in intimate attachment of the bacterium and enterocyte via re-arrangement of the intestinal cell architecture. These architectural changes result in loss of the microvilli, pedestal formation, and accumulation of the supporting cytoskeletal proteins beneath the bacteria that make the AE lesion visible under microscopy (Gyles 2007; Mota & Cornelis 2005; Kenny 2002).

## *Putative Virulence Factors*

Several products of the pO157 have been shown to contribute to the virulence of *E. coli* O157 infection. The magnitude of their contribution is likely small, which has made it difficult to definitively associate them with virulence using traditional epidemiologic approaches. Lim et al. (2010) proposed using the presence of the pO157 and comparing it to virulence as a proxy for the combined effect of its gene products. These authors then assert that the presence of the pO157 is associated with increased virulence. The pO157 contains genes responsible for the formation of several products: a hemolysin (ehxA), catalase-peroxidase activity (katP), a type II secretion system (etp), serine protease (espP), a metalloprotease (stcE), putative adhesion (toxB), and an Eae gene-positive conserve fragment (ecf; Lim et al. 2010). All of these products have been shown to contribute to a specific aspect of *E. coli* function or survival, primarily in *in-vitro* models. Individually, none have been associated with increased virulence in appropriate animal

models.  Recent work with a bovine model reported that absence of the pO157 in strains of *E. coli* O157:H7 decreased the survivability of the organism under acidic conditions and altered the propensity of it to colonize the rectal-anal junction (RAJ; Lim et al. 2007; Sheng et al. 2006).

An important virulence factor necessary for *E. coli* O157 to survive the first body defense in the human stomach is resistance to a pH of 1.5-3.0.  If it is able to survive the strongly acidic environment of the stomach chances of colonizing the lower gut increase.  Organisms that successfully evade destruction in the stomach employ an acid-tolerance response (ATR) system.  This system uses a glutamate decarboxylase and a gene encoded antiporter system to consume a proton and transport the product extracellularly, effectively maintaining a neutral pH in the cytoplasm (Thorpe et al. 2002).

Other factors not as well defined or understood that could also contribute to virulence are heat resistance, salt resistance, presence of EAST1 enterotoxin, presence of an adherence-conferring molecule (lha), and different regulators and effectors of the LEE (Lim et al. 2010).  The recent whole genome sequencing of two strains of outbreak associated *E. coli* O157 (Perna et al. 2001; Hayashi et al. 2001) and of the pO157 (Burland et al. 1998) greatly increased the list of possible putative virulence factors.

## *Epidemiology*

Cattle are the most important animal reservoir of *E. coli* O157:H7 in North America, whereas sheep are the most important animal reservoir in Australia (Gyles 2007).  Prevalence estimates for *E. coli* O157 shedding on cow-calf ranches have been quite variable and dependent on time of year and whether the calf population or the cow population was under study.  Gannon et al. (2002) found that 25% of calves were shedding *E. coli* O157 during the first 7 weeks of life; however, prevalence fell to 0% when calves were moved to pasture.   One week prior to

weaning, the shedding prevalence was 0-1.5%.  Two weeks post-weaning, the prevalence was 6-14%.  A similar trend was seen in the cows where 7 weeks post-partum *E. coli* O157 prevalence was 2-18% and then upon moving to pasture the prevalence fell to 0%.  Riley et al. (2003) found that, in a Southeastern US herd, 3% of samples from reproductive-age females were positive for *E. coli* O157; 9.1% of the cows sampled had at least one positive fecal test over a three month sampling window.

Sargeant et al. (2000) also showed low prevalence (1.3-1.9%) in cow-calf herds in Kansas.  This study also indicated that a calf whose dam was shedding *E. coli* O157 was not more likely to shed O157 than a calf from a dam never identified as shedding O157.  Conversely, an Australian dairy study estimated that a calf was 2.2 times more likely to shed VTEC if the dam was also shedding VTEC (Cobbold and Desmarchelier 2000).  Laegreid et al. (1999) reported only 6.9% of the cultures positive in a multi-herd, multi-state study; however, herd level prevalence was 87% with 63-100% seroprevalence to O157 antigen.  Shedding in a herd was positively correlated and high titers in serum. Furthermore, the force of infection was greatest in late summer and prior to weaning and declined during the first 6 weeks on feed.  In a Canadian study, farm-level *E. coli* O157 prevalence was 45% with an animal-level prevalence of 6%.  This study described significant risk factors associated with shedding and found that cattle supplemented with corn silage in the winter were 7.64 times more likely to shed O157 as cattle not supplemented with corn silage.  In summary, little cow-calf sector *E. coli* O157 epidemiology research has been done.  This is most likely due to the temporally long data collection process, low prevalence, erratic prevalence, unknown impact of shedding at the cow-calf stage on food safety, and greater interest directed at the feedlot and post-harvest sectors.

There have been a number of studies conducted examining the prevalence of *E. coli* O157 in feedlot cattle (reviewed in Rhoades et al. 2009). Fecal prevalence has ranged from 0.26% to 28% with considerable variation even within pen, depending on time of year and on sample processing procedures (Renter & Sargeant 2002). A large study conducted by Hancock et al (1997) indicated low animal-level prevalence (1.3% - 4.6%), moderate pen-level prevalence (13.3% - 52.5%), and high feedlot-level prevalence (61%; 61/100 feedlots). Although conducted in 13 states in the Midwestern U.S., no significant regional distribution of *E. coli* O157 shedding was identified. In contrast, Lejune et al. (2004) found a pen-level prevalence of 100% and a fluctuating animal-level prevalence ranging from 15% on arrival to the feedlot to 28% two weeks after arrival. Animal-level prevalence then decreased to 5-10% for the remainder of the study. Sargeant et al (2003) examined *E. coli* O157 prevalence in 73 feedlots located in four states, which represented approximately 15% of the fed cattle in the U.S. at the time of the study. They reported an overall *E. coli* fecal prevalence of 10.2%, pen-level prevalence of 52%, and a feedlot-level prevalence of 95.9%. Other studies reported animal-level prevalence to be 28% (Elder et al. 2000), 23% (Smith et al. 2001) and 15.7% (Chapman et al. 1997).

The true epidemiologic picture of *E. coli* O157 from discovery to present day has been difficult to contextualize as newer, more sensitive techniques for detection have been developed. One of the key differences between older studies that reported relatively low prevalence and more recent studies that reported greater prevalence is that the modern studies used immunomagnetic separation after the enrichment process. This step has reportedly increased the sensitivity of the combined culture technique by 100-fold (Chapman et al.1994). This has made it difficult to tell if there is a true increase in the prevalence, or an increase in diagnostic test sensitivity or both (Renter & Sargaent 2002) since the first reports in the 1980's.

Other studies have been performed to estimate the prevalence of *E. coli* O157 in other cattle production settings. Estimates from studies conducted outside of the U.S. on veal calves have estimated the prevalence of *E. coli* O157 to be low (0-9%) compared to adult cattle (16.1%-61.2%; Bonardi et al. 1999; Cobbaut et al. 2009; Heuvelink et al. 1998). In a recent longitudinal study conducted in Ontario on white veal calves, researchers estimated *E. coli* O157 calf-level prevalence to be 3.2%, while nearly 100% of the calves were positive for exposure to shiga toxin-producing organisms (Cristancho et al. 2008). Cobbault et al (2009) also compared prevalence of *E. coli* O157 between dairy farms and mixed dairy and beef farms. They reported that dairy farms had greater prevalence than beef and veal farms (61.2%, 22.7% and 9.1% for dairy, beef, and veal, respectively) and that the prevalence on mixed farms was significantly greater than that on beef farms (44.4% and 22.7% for mixed and beef, respectively). This is in contrast to Hancock et al. 1998 and Bonardi et al. 1999 who reported similar *E. coli* O157 prevalence between dairy and feedlot cattle.

Study of *E. coli* prevalence among different ages of adult cattle has produced mixed results. Some studies report that young adult cattle had greater prevalence of *E. coli* O157 compared to older adults (Van Donkersgooed et al. 1999; Hancock et al. 1997; Yilmaz et al. 2002). Conversely, Riley et al. (2003) found that older animals had greater *E. coli* O157 prevalence than did younger adults in a Texas study (13.5% and 8.2% for older and younger cattle, respectively). The effects of age on *E. coli* prevalence remain unclear. These factors are often confounded by production practices, geographic locations, breeds, and diet types (Rhoades et al. 2009).

The range of *E. coli* O157 prevalence estimates from hide and oral cavity samples from cattle varies widely (7.3% to 61.9%; Keen & Elder 2002; Barkocy-Gallagher et al. 2003; Rivera-

Betancourt et al. 2004; Elder et al. 2000; O'Brien et al. 2005). Such a wide range precludes

giving a single meaningful estimate of prevalence. There have been mixed results as to which

sample, fecal or hide, gives a more accurate estimate of true prevalence. Barkocy-Gallagher et

al. (2003) reported that hides had a greater prevalence of *E. coli* O157 than did fecal (60.6% and

5.9% for hide and fecal, respectively), whereas other researchers have reported the opposite

(Elder et al. 2000; Woerner et al. 2006). Reid et al. (2002) illustrated that location of the sample

on the hide may be an important factor in detecting *E. coli* presence. They isolated *E. coli* O157

from 22.2% of brisket samples but from only 4.4% of flank samples and 3.3% of rump samples.

This affirms comments by Elder et al. (2000) in which that they asserted that the brisket was the

location most likely to give a true reflection of animal-level *E. coli* prevalence because it

represented contamination from the bedding material in pens, whereas *E. coli* presence on flanks

and rumps might represent cross-contamination from pen mates during lairage. Other studies

examining only hide prevalence have reported relatively high (53% - 75.7%) *E. coli* O157

prevalence (Arthur et al. 2007; Arthur et al. 2004; Rivera-Betancourt et al. 2004). Hide

contamination of the carcass is generally accepted as one of the most important factors leading to

contaminated meat products (Edwards & Fung 2006).

The production and processing of meat products is governed by Chapter III under Title 9

in the Code of Federal Regulations (CFR). Section 417 in chapter III describes the points on

Hazard Analysis and Critical Control Points (HACCP; Hulebak & Schlosser 2002). The

implementation and refinement of HACCP at harvest plants has been largely responsible for the

significant reduction (i.e., over 40%) in the incidence *E. coli* O157-related illnesses in the U.S.

from 1996-2004 (CDC 2005). Harvest plant estimates are based on the location within the

fabrication process the samples are collected. The four commonly reported stages for sample

collection are pre-evisceration, post-evisceration, post-intervention, and chilled (Rhoades et al. 2009). Woerner et al. (2006) studied 15 lots of cattle from 12 feedlots were cultured at the feedyard and then followed through the fabrication process at the harvest facility. They reported fecal *E. coli* O157 prevalence of 24.7, 14.7, 27.6, 10.1, 1.4, and 0.3% for pen floor, hide, colon, pre-evisceration, post-evisceration, and final intervention samples, respectively. In that study, animals from pens with a pre-harvest fecal prevalence greater than 20% were associated with increased prevalence estimates in samples from the harvest facility; however, there was no difference in animals from low (<20%) prevalence pens. There appears to be a moderate correlation ($r = 0.67$) between positive fecal samples at the feedlot and positive hide samples at harvest. Elder et al. (2000) studied 341 carcasses and reported fecal, hide, pre-evisceration, post-evisceration, and final intervention *E. coli* O157 prevalence of 28, 11, 43, 18, and 2%, respectively. These authors also noted that there may be a relationship between fecal prevalence and carcass contamination. They suggested strategies enlisted on the farm may aid in the reduction of the risk of *E. coli* O157 infection from contaminated food.

Other studies have also reported low *E. coli* O157 prevalence on carcasses prior to evisceration, on carcass samples post-final intervention, on chilled carcasses, or in meat (Chapman et al. 2001; Barkocy-Gallagher et al. 2003; Arthur et al. 2004; Rivera-Betancourt et al. 2004).

### *Pre-harvest Control*

In a simulation study, Jordan et al. (1999) estimated that the greatest reduction in carcass contamination may result from vaccination with an agent that decreases the amount of *E. coli* O157 shed in the feces. This seems reasonable because of the strong relationship between fecal prevalence and carcass contamination (Elder et al. 2000; Loneragan & Brashears 2005). As a

result, a number of research groups have been focused on development and analysis of pre-harvest, farm-level interventions to decrease *E. coli* O157 fecal shedding in cattle. These interventions are designed to protect the meat supply and to decrease environmental contamination of soil and water that may be consumed directly or indirectly by humans.

The most intuitive method for the control of bacterial infections or bacterial colonization is the use of antimicrobials administered either directly to the animal or delivered to the animal in the feed. Ransom et al. (2003) reported a reduction of over 40% in cattle treated with neomycin alone, neomycin in combination with a probiotic, or a probiotic combined with a vaccine. It should be noted that the use of neomycin to control the fecal shedding of *E. coli* O157 in cattle is not approved by the FDA at the time of this writing. Also of significant concern is the perpetuation of antibiotic resistance to non-target species and their role in human-acquired infection. There is increasing pressure on animal agriculture to eliminate non-therapeutic uses of antibiotics; therefore, research investigating antibiotic administration to control *E. coli* shedding has been limited.

The effects of feed-grade ionophores on *E. coli* O157 shedding have been studied *in vitro* and i*n vivo* with no demonstrable effect (Edrington et al. 2003; Edrington et al. 2006). Two studies performed on cattle fed forage and corn diets were interpreted to suggest that there may be an interaction between diet and antimicrobial treatment (Van Baale et al. 2004; McAllister et al. 2006). These studies reported that there was a reduction in *E. coli* O157 shedding by cattle fed a forage-based diets but not by cattle fed a concentrate-based diet. Another closely-related approach is the use of bacterially-derived proteins such as colicins or bacteriocins that create a porous membrane in bacteria leading to death of the organism (Schamberger & Diez-Gonzalez 2002).

Other approaches to pre-harvest intervention involve feed probiotics or prebiotics. Probiotics are live microbial preparations that are added to the diets of cattle to improve the intestinal microbial balance in a manner that benefits the animal (Fuller 1989). Prebiotics are sugars and other organic compounds that are not processed or used by the host but are used by the resident intestinal microflora (Steer et al. 2000). The probiotic combination of *Lactobacillus acidophilus* and *Propionibacterium fruedenreichii* has reduced *E. coli* O157 fecal shedding from 27% to 16% and hide prevalence from 14% to 4% under field conditions. There also appears to be an improvement in growth efficiency that may offset product cost, making this probiotic a possible component in a complete *E. coli* O157 reduction program (Elam et al. 2003; Younts-Dahl et al. 2004; Stephens et al. 2007). To date, no cost-effective strategies using prebiotic feed additives in food animals have been reported(Oliver et al. 2009). Using a similar approach known as competitive exclusion, Zhao et al (1998) demonstrated a reduction in *E. coli* O157 by feeding a mixture of non-O157 E. coli.

The effects of diet on *E. coli* shedding has been extensively studied (reviewed in Callaway et al 2009). Cattle on barley-based diets had greater fecal prevalence and increased quantity of *E. coli* O157 than cattle on corn-based diets (Berg et al. 2004). Cattle fed a steam-flaked corn diet had increased *E. coli* O157 prevalence compared to cattle on a dry-rolled corn diet (Fox et al. 2007). This has led to the theory that grain types or grain-processing methods that increase fermentable carbohydrate reaching the distal intestine will increase the amount of colonic VFA available and will increase the *E. coli* O157 population. Tkalcic et al. (2000) compared fecal prevalence of *E. coli* O157 among forage-fed cattle and grain-fed cattle after inoculation with O157. They reported that grain-fed cattle consistently had a greater fecal prevalence than that of forage-fed cattle. Similarly, a series of projects examining the effects of

distiller's grains (wet and dry) on *E. coli* O157 fecal prevalence found that cattle fed diet

containing distiller's grains usually had increased fecal prevalence of O157 (Jacob et al. 2008a;

Jacob et al 2008b). It is important to note that the difference in fecal prevalence was only at the

individual-animal level whereas there was no difference in the pen-level fecal prevalence and

only at a single time point (d 122), with no difference at the final sampling time point at the

animal or pen-levels. It is difficult to say with certainty how feeding distiller's grains may affect

fecal *E. coli* O157 shedding. Forage feeding reduced fecal *E. coli* O157 shedding in cattle but

reduced live bodyweight (Callaway et al. 2009).

Another intuitive method for control of bacterial infection and colonization is through the

development and use of an effective vaccine. Two different vaccines have been developed and

tested in production settings in cattle. The first vaccine is based on the type III secretory proteins

(TTSP) that are key components in attachment to the intestinal epithelial cell. Potter et al. (2004)

demonstrated a 58.7% lower risk of recovering *E. coli* O157 in the feces of vaccinates over

controls. Moxley et al. (2009) then followed with a dose determination study and reported a

65% reduction in the risk of recovering *E. coli* O157 from feces of cattle vaccinated three times

verses controls. The second vaccine that has been developed is based on Siderophore Receptors

and Porin proteins (SRP) that are involved with bacterial iron acquisition (Chakrabroty et al.

2007; Stevens & Thomson 2005). This vaccine manufactured by Epitopix (Willmar, MN)

received conditional licensure in 2009 in the U.S. Thornton et al. (2009) in an experimental

inoculation study demonstrated a reduction in the number of vaccinated cattle shedding *E. coli*

O157 in the feces and a tendency towards a reduction in the concentration of O157 when they

were shedding. Similarly, Thomson et al. (2009) in a field study found that vaccinated cattle had

an 85.2% reduction in risk of shedding *E. coli* O157 in the feces and a 98.2% reduction in the

concentration of fecal E. coli.  Wileman et al. (2010) also examined the response of calves born

to cows that received the vaccine prior to calving and found that passive transfer of *E. coli*

O157:H7 specific antibody does occur under field conditions in beef cattle.  An effective vaccine

developed for on-farm control of E. coli O157 could have additional benefits extending to other

food sources from decreased environmental contamination (Jordan et al. 1999; Elder et al. 2000).

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# CHAPTER 2 - Analysis of modern technologies commonly used in beef cattle production: Conventional beef production verses non-conventional production using meta-analysis

**Abstract:** Conventional feeding systems use pharmaceutical products not allowed in natural or organic systems for finishing cattle. This review of data compares the performance effects (ADG, G:F, DMI) of technologies used in conventional feeding programs that are prohibited in organic and/or natural programs. The technologies evaluated were steroid implants, monensin, tylosin, endectocides, and metaphylaxis with any antimicrobial. For inclusion in this analysis, studies were conducted in North America; reported randomization to treatment group; utilized beef cattle; contained an untreated control group; and were sourced from peer reviewed journals. Forest plots were used to visually examine the data for trends towards a uniform effect of the technology on the outcomes of interest (ADG, DMI, G:F). Technologies that displayed a uniform response compared to negative controls on the forest plot were then analyzed using mixed models. Examination of forest plots for endectocides, steroid implants, monensin and metaphylaxis technologies appeared to show performance advantages for treated cattle relative to cattle in negative control groups. An insufficient number of studies met the inclusion criteria to conduct meta-analyses comparing endectocides, monensin or tylosin to negative controls. Average daily gain in feeder cattle given metaphylaxis on arrival was 0.11 kg/d ($P < 0.01$) greater relative to cattle that did not receive metaphylaxis on arrival. Implanting heifers increased ADG by 0.08 kg/d compared to non-implanted controls ($P = 0.09$). Implants had no effect on G:F ($P = 0.14$) in heifers or on DMI ($P = 0.44$) relative to non-implanted control heifers. Implanting steers was associated with greater ADG by 0.25 kg/d ($P < 0.01$) and DMI by

0.53 kg/d ($P < 0.01$) relative to non-implanted control steers. Implants also improved G:F in steers relative to non-implanted steers by 0.02 (0.17 vs. 0.15; implanted vs. controls, $P < 0.01$) (n = 21 studies). When average estimated differences in ADG and G:F for implanted and non-implanted steers were incorporated into a breakeven model, implanted steers had a $77 per head lower cost of production than non-implanted steers and $349 per head lower cost of production than organically raised steers. These data illustrate the importance of capturing premiums when operating natural and organic production systems to maintain economic viability.

Keywords: Beef cattle, conventional, finishing performance, meta-analysis, non-conventional

**INTRODUCTION**

There is an increasing focus on organic and natural beef production in the United States. Conventional feeding systems use pharmaceutical products not allowed in organic systems for finishing cattle and do not utilize certified organic feedstuffs as defined under the national organic program (NOP, 2008). The recent USDA regulations for natural beef production prohibit the use of growth promoting hormones and animals that have received antimicrobials. However, the new rule does allow the use of ionophores for the control of coccidiosis (USDA/AMS 2009). Until recently there has been no standard definition of "natural beef production" which has lead to inconsistencies between "natural" beef production systems. There is very limited research comparing an entire beef cattle production system with pharmaceutical technologies versus one that excludes those technologies (Fernandez and Woodward, 1999; Sawyer, 2003; Berthiaume, 2006), thus limiting the ability to accurately predict the performance differences between cattle in the different systems. Contrary to a review article, a meta-analysis

provides a method of examining existing literature critically and in a quantitative manor, accounting for within and between trial variance, in order to provide an overall estimate of effect of a given question(s) based on existing data. The aim of this study was to evaluate the performance effects (ADG, DMI, and G:F) and health effects (morbidity and mortality) of pharmaceutical technologies used in feedlot cattle that may be routinely excluded from non-conventional production systems. This report uses the techniques of forest plot analysis and meta-analysis to evaluate the technologies of steroid implants, monensin, tylosin, endectocides, and metaphylaxis with any antimicrobial on arrival and their effects on the performance and health of feedlot cattle. In addition, liver abscess risks of cattle receiving tylosin verses cattle not receiving tylosin were also examined.

## MATERIALS AND METHODS

### *Data Gathering*

The question was: What is the difference in ADG, G:F, DMI, morbidity, mortality and liver abscess risks in feedlot cattle with and without pharmaceutical technologies in North America? Manuscripts were identified through PubMed, and Commonwealth Agricultural Bureau (CAB) electronic databases from February 2008 thru April 2008. A variety of search terms were used to identify articles that possibly contained relevant information about the review questions (Table 1). Each search contained at least one search term from each category. After retrieving the citations, each title and abstract was read by the first author and retained for further evaluation if it mentioned at least one of the treatments and at least one of the outcomes of interest in beef cattle. Further, the Intervet Schering Plough Animal Health (Kenilworth, NJ) and Texas Tech University North American TBA Implant Database (TTU Implant Database, 1999) was used to identify studies utilizing steroid implants. The information contained in the database is

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searchable. The manuscripts and technical bulletins from which the data were sourced are linked from the site. All articles were cross-referenced with abstracts obtained through the PubMed and CAB database searches, and technical bulletins from implant database were included as well. These manuscripts and technical bulletins were assessed using the same inclusion criteria except for being from peer reviewed sources. Only data from studies using a single implant treatment (i.e. no re-implant) with a contemporary non-implanted control group were considered relevant. The implant data was further sub grouped into implant studies utilizing heifers and studies utilizing steers to attempt to mitigate the amount of variability in the data sets.

After identifying relevant studies based on the title and abstract, the complete manuscript was obtained and critically evaluated. Studies were only retained for further consideration if the study was conducted in North America, used randomization to allocation of treatment group; used beef breed animals and contained an untreated control group. Manuscripts that did not meet all of the above criteria were excluded from further consideration. Data were then extracted from the remaining studies. Data was extracted by recording the point estimates for ADG, DMI, G:F, mortality, morbidity, presence of liver abscess and corresponding **SE** for each treatment group, description of experimental unit, number of experimental units, and sex of cattle for each of the manuscripts.

For studies that reported results for different dose levels (i.e. monensin 100, 200, 300 mg/hd) the average treatment effect was calculated via a calculated mean of the treatment responses from the data at the individual dose levels. This data extraction approach was required for five studies in which authors were not able to separate out the information and report it separately. The measure for SE of the treatment effects in these studies was a pooled SE as reported in the manuscript. When studies reported a pooled SE, this was converted to a standard deviation by

multiplying the pooled SE of the difference in sample means by the square root of the number of experimental units. The number of experimental units depended on what was defined as the experimental unit, the animal or the pen i.e. for 2-arm parallel comparison with 100 animals in 10 pens per arm and treatment allocated at the pen level , then n = 20. The standard deviation was then squared to obtain the variance, which was used in the meta-analysis.

### Data Analysis

Forest plots using the R-statistical package and the rmeta and meta packages (R Development Core Team, Vienna, Austria) were used to visually assess if the effect of the technology on the outcomes of interest (ADG, DMI, G:F) was uniform across studies. For this graphic approach, the production outcome and standard deviations were used to calculate the difference between groups and these data used for the forest plot. In a forest plot each study is listed on the left hand side of the graph individually. The horizontal line listed next to each study represents a 95% confidence interval for the difference between groups for continuous outcomes. The size of the shaded box in the middle of the horizontal line represents the relative weight of a study compared to the other studies. The weight is a reflection of the number of experimental units involved in the study, i.e. the greater the number of replicates the greater the weight. All of the studies are then oriented in relation to the large vertical line listed as the "zero" line or no effect line. This line represents a situation in which the difference between the two treatments equals zero. Studies to the right of the null value indicate a positive value and studies to the left have a negative value. Technologies were considered to be uniform by the first author when 50% of the point estimates (boxes) on the forest plot were to one side of the null effect line.

For the continuous outcomes ADG, DMI and G:F, technologies considered to display a uniform response compared to negative controls were analyzed using general linear mixed models in Proc Mixed of SAS (SAS Inst. Inc., Cary, NC). In brief, each model of continuous outcome variables (ADG, DMI, G:F) contained one fixed effect (steroid implants, monensin, tylosin, endectocides, metaphylaxis), a random intercept effect for each study, and a repeated effect to incorporate a within-study variance for each study (van Houwelingen 2002, St. Pierre (2001). To define the covariance parameter for the between-study variance (range: 0.01 to 1.0 by 0.01), values for the within-study variance for each study (extracted from the literature) were used to create the profile likelihood function and resultant 95% confidence interval for the between-study variance (van Houwelingen 2002). The only change from the form described by van Houwelingen (2002) was the addition of the least squares means command to generate model-adjusted means and SE for fixed effects and establish a single overall treatment effect for each technology that showed a statistically significant difference (alpha < 0.10). The model-adjusted means and SE for ADG, DMI and G:F, when statistically significant, were used to derive a 95% confidence interval for the summary effect and incorporated into a standardized feedlot breakeven model.

The metaphylaxis morbidity and mortality data and the tylosin liver abscess incidence data were analyzed with generalized linear mixed models with a logit link and binomial distribution using the Glimmix procedure of SAS (SAS Inst. Inc., Cary, NC). The outcome variables were modeled in an events/trial format where the denominator represented the total number of cattle within a group and the numerator included only those with the outcome of interest (morbidity, mortality, liver abscess). A repeated statement was used to account for the multiple observations within studies, and a random intercept term was used to account for the potential correlations

among groups within studies. Metaphylaxis was included in the model statement as a fixed effect, and when significant, model adjusted estimates were transformed from the logit form to generate the estimated probability of treatment or death if receiving metaphylaxis, or having liver abscesses if consuming tylosin, respectively. Therefore, the estimated probability represents a cumulative incidence or risk of these adverse health events occurring at some point during each trial.

### Breakeven Model

A standardized feedlot breakeven model commonly used by feedlot consultants and managers to assess the economics of feeding a pen of cattle with user defined inputs was used (Thomson, 2008). The economic model assumptions are listed in Table 2. Based on an average of the ADG and G:F reported in Berthiamune (2006), Fernandez and Woodward (1999), and Sawyer (2003), we used an ADG of 1.30 kg/d and a G:F of 0.14 to predict the breakevens for "natural" raised calves. The model was then used to simulate implanting the "natural" calf and the estimated differences in ADG and G:F from the meta-analysis were used to estimate a breakeven. And finally, the model was used to predict feeding the "natural" calf an organic diet. For the organic breakeven, the days on feed was increased by 20 **d** (Fernandez and Woodward, 1999) and the organic feed costs were multiplied by 1.5 (USDA, 2003) to simulate the feedlot performance and feed cost differences of organic cattle. A sensitivity analysis was performed for organic feed prices ranging from 1.25 to 1.75 of conventional feed prices.

### RESULTS

A total of 14,311 citations were identified by the initial electronic search. After examining the titles and abstracts for possible relevance and removing duplicate citations, 140 manuscripts

were retrieved for quality assessment and data extraction. After quality assessment and application of inclusion criteria, 91 treatment to control comparisons were identified from 51 manuscripts (Table 3). The three most frequent reasons studies were excluded were failure to include an untreated control group, failure to report variation of the outcome either as a standard deviation or SE, and failure to use randomization to allocate animals to treatment groups.

Based on visual assessment the forest plots, use of endectocides (Figure not shown), steroid implants (Figures 1 and 2), monensin (figure not shown) and metaphylaxis (Figure 3) showed a performance advantage for treated cattle relative to the cattle in negative control groups, i.e. more than 50% of point estimates to the right of the null. Tylosin studies did not show a consistent advantage in treated cattle relative to control cattle with respect to ADG, G:F and DMI. An insufficient number of studies met the inclusion criteria to conduct a meta-analysis comparing endectocides, monensin or tylosin. Therefore, a summary effect measure was only calculated for metaphylaxis and implant data sets.

### Meta-analysis and Breakeven

Average daily gain in feeder cattle receiving metaphylaxis using a variety of antibiotics on arrival was 0.11 kg/d (95% CI = 0.10 to 0.13, $P < 0.01$) relative to cattle that did not receive metaphylaxis on arrival (Table 4). The use of implants in heifers was associated with increased ADG by 0.08 kg/d compared to non-implanted controls (95% CI = 0.01 to 0.15, $P= 0.09$). The use of implants in heifers was not associated with differences in G:F ($P = 0.14$) or DMI ($P = 0.44$). The use of implants in steers was associated with 0.25 kg/d greater ADG (95% CI = 0.23 to 0.27, $P < 0.01$) and 0.53 kg/d greater DMI (95% CI = 0.45 to 0.61, $P < 0.01$) relative to non-implanted control steers. The use of implants was also associated with increased G:F in steers

relative to non-implanted steers by 0.02 (0.17 vs. 0.15; implanted vs. controls, 95% CI = 0.018 to 0.022, $P < 0.01$).

The point estimates of differences in ADG and G:F for implanted and non-implanted steers were incorporated into the breakeven model. The model suggests that implanted steers were associated with $77 per head lower cost of production than non-implanted steers fed similar diets. Also, implanted steers fed a non-organic diet had a $349 per head lower cost of production than non-implanted cattle fed an organic diet assuming being sold on the same market. The sensitivity analysis performed on the assumption of organic feed price being 1.5 of conventional feed prices. These results indicated that the cost of production for organic beef, as well as conventional, is highly sensitive to feed price. For each 10% increase in the price of organic feed, the breakeven estimate increased approximately $54/head. The simulations did not incorporate morbidity and mortality effects.

### *Morbidity, Mortality and Liver Abscesses*

For cattle that received metaphylaxis using a variety of antibiotics upon arrival to the feedyard, morbidity was estimated at 29% (95% CI = 21.2% to 38.58%) compared to 55% (95% CI = 44.46% to 65.14%) in the cattle that did not receive metaphylaxis ($P < 0.01$; Figure 4). For cattle receiving metaphylaxis on arrival to the feedyard, mortality was estimated to be 1.8% (95% CI = 1.05% to 3.12%) compared to 3.8% (95% CI = 2.30% to 6.50%) for cattle not receiving metaphylaxis ($P < 0.01$; Figure 4). Cattle not fed a ration containing tylosin had an estimated 30% (95% CI = 18.62% to 44.77%) risk of having liver abscesses compared to 8% (95% CI = 4.43% to 14.07%) in cattle consuming tylosin ($P < 0.01$; Figure 4).

### DISCUSSION

This study suggests performance effects for several modern technologies on North American beef cattle production. Studies that compared a single implant to no implant in steers indicated an improvement in ADG by approximately 0.25 kg/day and improved G:F by 0.02. Over a 210-d feeding period this could result in a 52.5 kg live weight difference between implanted and non-implanted steers. This is approximately a 17% improvement in ADG and a 9% improvement in G:F which is in agreement with other reports of expected implant performance when compared to non-implanted cattle (Bartle, 1992, Preston 1999, Duckett and Andrae 2001).

Science based performance and economic expectations can be achieved by using the meta-analysis performance results in a breakeven model. These data can provide an accurate indication of the premiums necessary to offset increased costs of producing beef in alternative systems compared to producing beef in conventional beef production systems. The use of metaphylaxis on arrival resulted in an estimated 53% reduction in subsequent morbidity treatments and an estimated 27% reduction in mortality losses compared to cattle not receiving metaphylaxis.

Feeding tylosin to feedlot cattle reduced the liver abscess risks from 30% to 8% in the studies examined. This study did not look at the severity of liver abscesses and relate it back to subsequent performance. This might explain why there was no significant difference in ADG between tylosin treatment groups. Nagaraja and Lechtenburg (2007) in their review of liver abscesses reported significant variation in the performance effects of abscessed livers and stated that it was likely a function of severity. The small or mild liver abscesses likely have less of a negative impact on performance than do larger or more severe abscesses.

In general, the use of these technologies has increased the amount of beef produced per animal and has produced that beef more efficiently and economically (Lawrence and Ibarburu,

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2006). The benefits of using these modern technologies are not just limited to the three performance indicators that we measured. There are additional impacts, such as control of bloat, coccidiosis, and external parasites, which occur with use of technologies but were not included in this analysis due to a dearth of published data. There are still other technologies that were not addressed in this study that are commonly used to control the reproductive cycling in heifers or increase the amount of HCW in the final period of feeding. As technologies are integrated into conventional feeding systems, their effects on economical and biological efficiencies in beef cattle production, as well as their effect on environmental and animal welfare issues will need to be examined.

This meta-analysis was broader in scope than some of the previously published meta-analyses in beef cattle production (Van Donkersgoed, 1992; Wellman and O'Conner, 2007). Most meta-analyses try to answer the question, "Is one treatment more favorable than another in terms of a single or small number of outcomes?". McPhee et al. (2006) demonstrated the use of meta-analysis for applications examining multiple treatments with multiple outcome effects. The current study was designed to examine the effects of one production practice, which has multiple treatments and multiple potential effects, as compared to another production practice that excludes all of the treatments. This by default introduced a large amount of heterogeneity into the meta-analysis which would preclude reporting any summary of effect statistics used in traditional meta-analyses. There was no test for homogeneity done on the data sets. The existence of heterogeneity was assumed, and was the basis for the use of the random effects model as was described previously. Van Houwelingen (2002) stated that, "heterogeneity might be present and should be part of the analysis even if the test for heterogeneity is not significant."

St. Pierre (2001) also remarked that the use of the random effects mixed model provides a more accurate estimate of values by allowing for the random effect of different studies.

One major source of bias is the approach used to identify studies. Published studies and technical reports are strongly subject to publication bias. There is a potential likelihood that studies which failed to show an effect have decreased opportunity to be published. Therefore, the source of papers may represent a biased subset of papers and if this is the case, may provide a best case scenario difference. Unfortunately, it is not possible to estimate the magnitude of publication bias that may or may not exist. Further, it is feasible that for "truly effective treatments" publication bias may not occur for different reasons – i.e. if a treatment is truly effective the need to continue to publish the same outcome many times is diminished. The consumer of the review must make their own decision about the potential for publication bias and its impact.

Summary effects were reported in this study for two main reasons. First, the heterogeneity in the meta-analysis was limited as much as possible for such a broad question through the critical manuscript review and inclusion criteria, and the remaining heterogeneity reflects the reference population for which the models seek to represent. There is a significant amount of variation between different segments of the beef cattle industry in terms of cattle management and production system goals. One producer may use a single implant, while others may use up to four in a calf's lifetime. Secondly, this study will hopefully stimulate more quantitative research on the health and performance effects of natural and organic beef production.

None of our summary statistics disagreed with smaller related reports in the literature. In addition, our summaries seem to agree with anecdotal responses seen in the field. Based on a study of just 54 cattle, Fernandez and Woodward (1999) reported that a 39% greater selling price

would be required to compensate for the performance reductions incurred with organic beef production as compared to conventionally produced steers. Our simulated breakevens indicate that a $0.62/kg live BW premium is required for an "organically-raised" animal to generate the equivalent net return compared to a "conventionally-raised" animal. Fernandez and Woodward (1999) also reported a 0.03 decrease in G:F and a 16% (0.26 kg/day) decrease in ADG in "organically-raised" steers compared with "conventionally-fed" steers. Our study also found that the difference in feed cost makes up the majority of the difference in costs between the two systems. In a 40-head study, Berthiaume et al (2006) found that non-implanted cattle had a 16% reduction in HCW, a 31% reduction in quality grade and would require a 15% greater premium over implanted cattle to remain equivalent. Our simulated breakeven model suggests that a "naturally" raised steer will have to generate at least a $0.14/kg live BW weight premium to generate an equivalent return as a conventionally raised steer. Sawyer et al. (2003) also demonstrated similar results in 64-steer study. Lawrence and Ibarburu (2006; 2008) also used meta-analysis to examine the effects of removal of all pharmaceutical technologies from all segments of beef production. There were no statements as to article selection or the process for examining the evidentiary value of the articles. Nonetheless, they estimated that the affect of removal of pharmaceutical technologies from the feedlot phase of production to be $155/hd which is similar to the estimate calculated from this study. Our model only examined the effects of a single implant and used only the difference due to that single implant in the analysis. Lawrence and Ibarbaru attributed $71 of the $155/hd difference to implants whereas this analysis estimated $77/ hd benefit. There are likely benefits in ADG and G:F for multiple implant programs that could explain the remainder of the difference between our estimates and those of Lawrence and Ibarbaru.

The primary purpose of a meta-analysis is to critically evaluate existing literature and provide an overall summary of effect. This report was able to provide an overall summary of effect for a limited number of the technologies. The secondary purpose of a meta-analysis is to highlight reasons why studies were not found and/or included in the analysis. For this report the secondary purpose may be as important as the primary purpose. We found that there has been a shift away from including untreated controls in many studies in an effort to compare one technology to another. As the natural and organic industries continue to grow, it will be important to evaluate the effect of various technologies on beef production efficiency. It is also important that the beef industry conducts further field trials comparing natural or organic systems directly to conventional systems. It is equally important to report the data more thoroughly by reporting measures of variation between treatment groups and between measured outcomes, and accurately describing methods used for blinding as we had to exclude several studies for these reasons. It is possible the analysis presented here overestimates the direct impact of these technologies due to publication bias. However, it is also likely that these products are highly effective as they are widely adapted in conventional production systems.

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**Figure 1**: Forest plot of implanted steers and corresponding ADG: overall mean effect and 95% confidence interval listed by the single implant used in the study.  All are referenced from Texas Tech implant database.

**Figure 2**: Forest plot of implanted steers and corresponding DMI: overall mean effect and 95% confidence interval listed by the single implant used in the study. All are referenced from Texas Tech implant database.
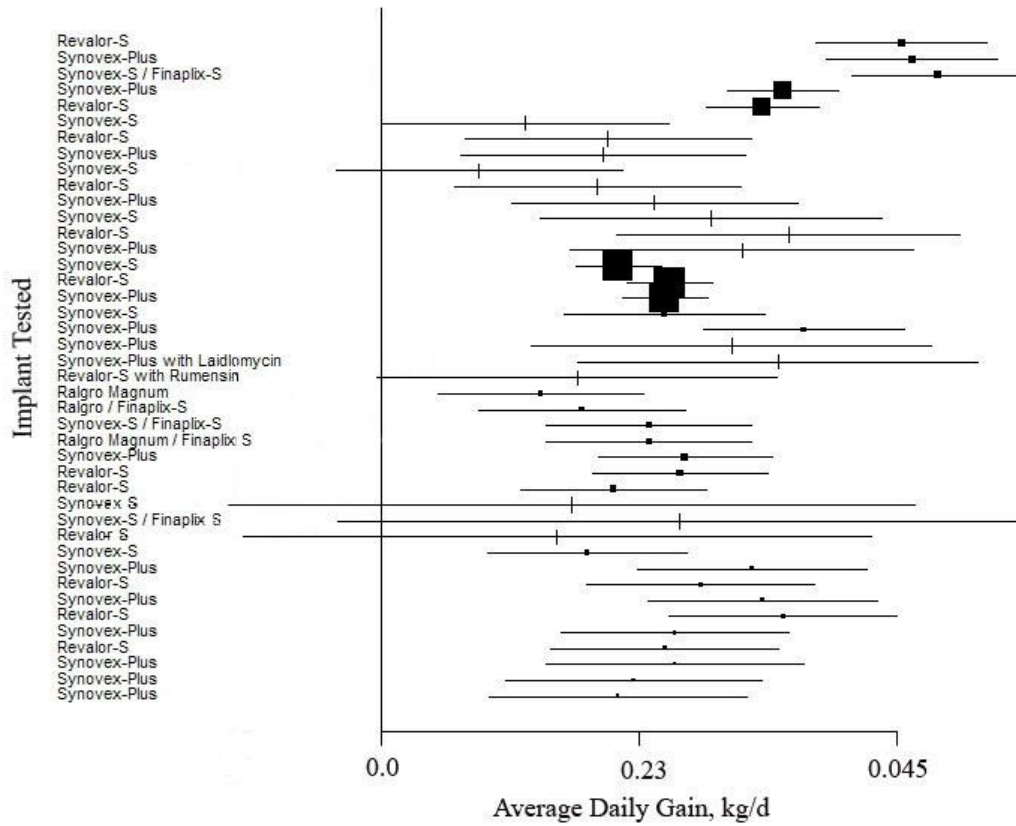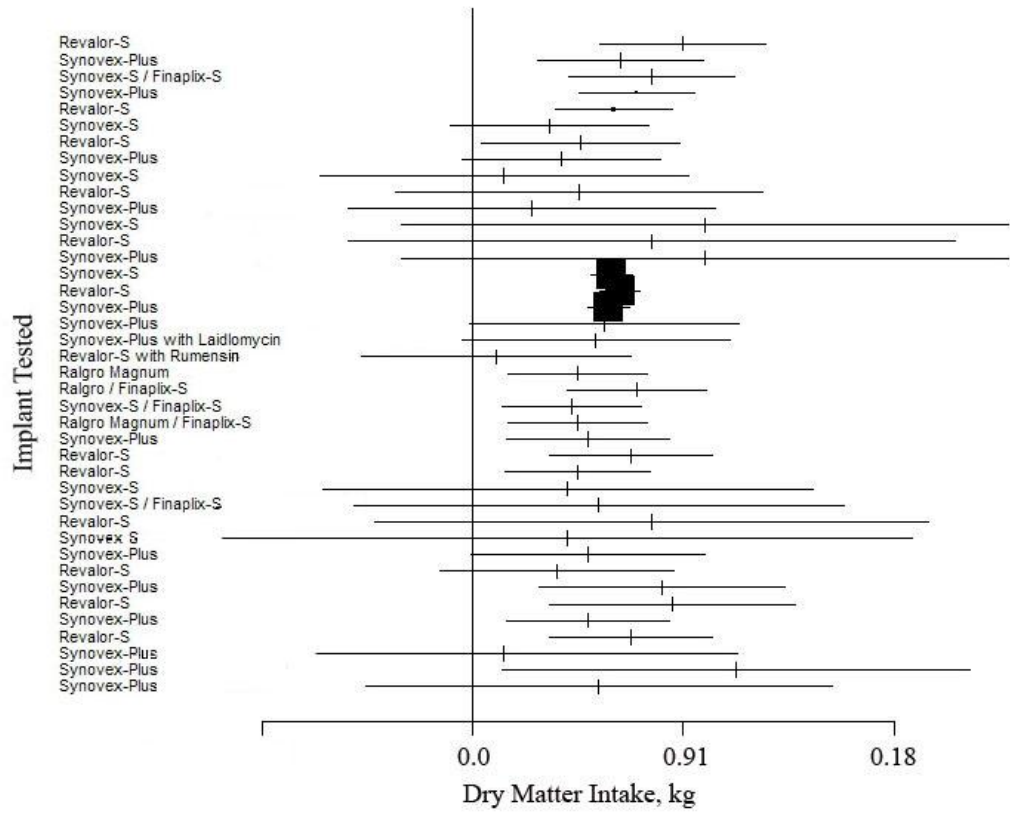
Dry Matter Intake, kg

**Figure 3**: Forest plot of metaphylaxis use and corresponding ADG: overall mean effect and 95% confidence interval listed by manuscript and study.
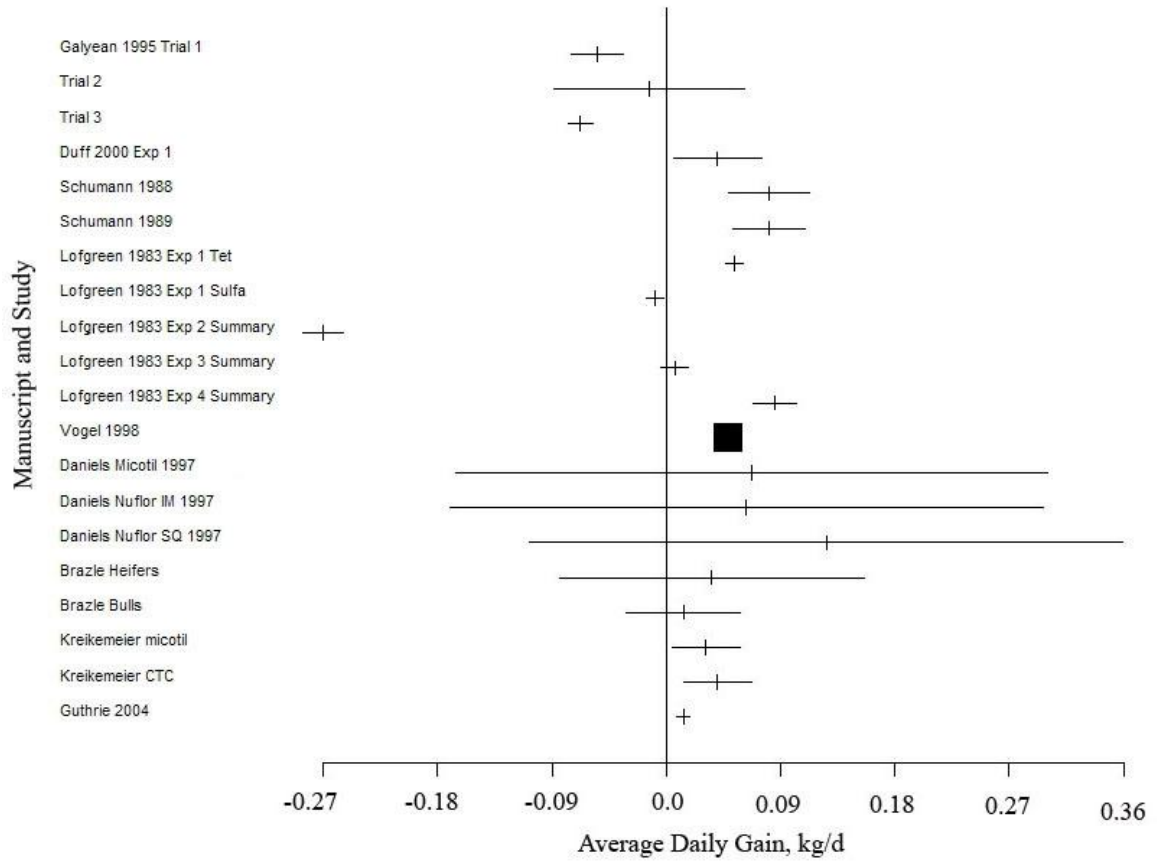
**Figure 4**:  Risks of treatment and death of cattle receiving and not receiving

metaphylaxis and risks for liver abscesses in cattle receiving and not receiving tylosin
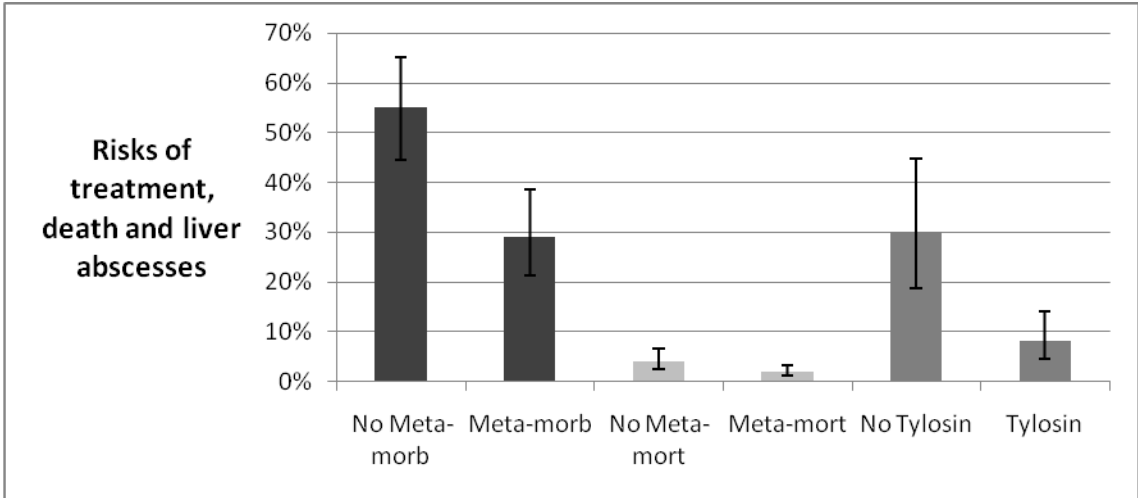
Table 1: Search terms used in PubMed and CAB databases. A word from each category was selected and combined with the word "and" to form search phrases to identify any relevant manuscripts.

| Population | Treatment | Outcomes |
|---|---|---|
| Beef | Metaphylaxis | ADG |
| Cattle | Prophylaxis | Average Daily Gain |
| Cow | Mass Medication | FE |
| Bovine | Antibiotic | Feed Efficiency |
| Steer | Ionophore | DMI |
| Heifer | Monensin | Dry Matter Intake |
| Calf | Rumensin® | Gain to feed |
| Calves | Tylosin | Morbidity |
| | Tylan® | Mortality |
| | Implant | Treatment |
| | Steroid Implant | Retreatment |
| | TBA | Abscess |
| | Estradiol | Liver abscess |
| | Estrogen | |
| | Endectocide | |
| | Ivermectin | |
| | Fenbendazole | |
| | Pour-on | |
| | Parasiticide | |

Table 2:  The economic model assumptions used for the common breakeven inputs.
Dollar amounts are in US dollars.

| Economic model | Assumption |
|---|---|
| In BW (kg) | 250 |
| Laid in price ($/kg) | $2.42 |
| Vaccination cost | $2.50 |
| Dewormer Cost | $1.60 |
| Feed Cost ($/909kg) | $209.00 |
| Days on Feed | 210 |
| Yardage/day $•hd⁻ | $0.30 |
| Interest rate | 6.50% |
| Out BW (kg) | 568 |
| Shrink | 4.00% |
| Fat Cattle Price/kg | $2.11 |

Table 3:  The total number of studies used in the meta-analysis listed by the technology
that was investigated.

| Technology | Number of Studies Included |
|---|---|
| Metaphylaxis | 20 |
| Implanted Heifers | 9 |
| Implanted | 44 |
| Monensin | 6 |
| Tylosin | 6 |
| Endectocides | 8 |

Table 4: Differences in least squares means ± SEM for Implants and Metaphylaxis from

significant results in the mixed model

| Technology | ADG (kg/day) | Improved FE | DMI (kg/day) |
|---|---|---|---|
| Metaphylaxis | 0.11 ± 0.02*** | --- | --- |
| Implanted Heifers | 0.08 ± 0.04† | 0.01 ± 0.003‡ | --- |
| Implanted Steers | 0.25 ± 0.01*** | 0.02 ± 0.001*** | 0.53 ± 0.05*** |

‡ P= 0.14
† P<0.10
*** P<0.001

# CHAPTER 3 - Characterization of Specific Passive Immunity Stimulated by Vaccination of Beef Cows Grazing Native Range with *E. coli* O157:H7-SRP®Abstract

**Abstract**

Twenty, four year old, mixed breed cows were selected from the Kansas State University commercial cow-calf herd for this study. Cows were assigned randomly to one of two treatments, placebo or *E. coli* O157:H7 SRP®[a] vaccination prior to calving. Serum total protein (TP) and serum *E. coli* O157:H7 SRP® antibody levels (SRP) were measured pre-suckle and at set intervals post-suckle in calves. All 20 cows were negative for fecal shedding of *E. coli* O157:H7 throughout the study. Vaccination treatment that cows received had no effect on calf serum TP level ($P > 0.05$). However, length of time post-birth had a significant effect on calf serum TP levels ($P < 0.001$). Failure of passive transfer was recognized as early as 6 hours post-birth. There was a vaccine treatment by time post-birth interaction observed for calf serum SRP® antibody levels ($P < 0.01$). Calves born to dams vaccinated with *E. coli* O157:H7 SRP® vaccine had elevated serum SRP antibody titers over time compared to placebo vaccinated calves. This study revealed that failure of passive transfer can be confirmed as early as 6 hours post-birth when colostrum supplements could still be administered. Also, this study demonstrated that successful *E. coli* O157:H7 SRP® antibody passive transfer can occur in beef calves under natural range conditions. This could be the first step of understanding lifecycle immunization strategies against *E. coli* O157:H7 in cattle and its effects on shedding of the organism by the animal at the point of harvest.

**Introduction**

Calves are born agammaglobulinemic due to the separation of fetal and maternal blood flow that prevents the transfer of immunoglobulins from cow to fetus in utero.[2] Following parturition the calf is reliant upon the immunoglobulins contained in the colostrum for protection from disease challenge during the first 2-4 weeks of life.[3] Colostrum feeding has been reported as one of the most important management practices to prevent mortality during the first 21 days of a calf's life.[22] Calves that fail to consume an adequate amount of colostrum within the first 12-24 hours after birth are at a higher risk for disease, death and decreased performance.[23,24] A number of studies have been performed examining the ability to passively transfer specific antibodies to beef calves, but none have examined *E. coli* O157:H7.[7,10,14,20]

The epidemiology of *E. coli* O157 is not well understood and the prevalence of *E. coli* O157:H7 in cow-calf herds has not been thoroughly studied. Some researchers have estimated the beef cattle herd level prevalence of *E.coli* O157:H7 to be 80-100%.[8,11,15,17] However, individual animal level prevalence seems to be much lower, 2-18%.[8,11,15,16] Designing the most effective *E. coli* O157:H7 control strategy will require knowledge of environmental risks and best management practices that have an impact on infection/colonization of the animal with *E. coli* O157:H7. Currently the majority of control strategies for *E. coli* O157:H7 are aimed at the reduction of pathogen load at the feedyard level or post-harvest.[12,16] Two studies utilizing beef cattle have suggested that initial infection of beef calves with *E. coli* O157:H7 may occur from their dams during the immediate post-natal period.[8,11] Therefore, current control strategies that target the feedlot and harvest phases of the production system are combating a post-colonization issue. A method to greatly lessen or eliminate the initial colonization of young beef calves would be a better food safety solution.

A new *E. coli* O157:H7 SRP® vaccine has been issued conditional licensure for use in feedlot cattle in the United States.[5,19] It is assumed that most circulating immunoglobulins will be concentrated in the colostrum of cattle.[9,13,21] Therefore, the use of this vaccine with its unique epitopes and mode of action warrants further investigation into its ability to achieve passive transfer to possibly eliminate or decrease colonization of *E. coli* O157:H7 in beef cattle. The objective of this study was to determine if *E. coli* O157:H7 SRP® specific antibodies could be detected in the serum of calves that consumed colostrum from dams that were vaccinated before parturition with *E. coli* O157:H7 SRP® vaccine.

**Materials and Methods**

*General Overview*

In January of 2009, 20 cows were utilized from the Kansas State University commercial cow-calf herd to study the efficacy of passive transfer of specific *E. coli* O157:H7 SRP® antibodies in beef calves. Four year old cows were selected, to control for parity and age, from the herd based on predicted calving dates and were balanced across pastures. Cows were maintained on native dormant bluestem pasture and were supplemented 6 days per week with a soybean meal based supplement. Cows were assigned randomly to one of two treatments: placebo or *E. coli* O157:H7 SRP® vaccination. Cows were vaccinated with their assigned vaccine treatments at 60 and 30 days prior to the projected start of the calving season. Blood samples were collected from cows prior to the initial vaccination to ensure an *E. coli* O157:H7 SRP® antibody free status. Serum ELISA's for detection of *E. coli* O157:H7 SRP® antibodies were conducted by Epitopix, LLC (Willmar, MN). All laboratory personnel were blinded to treatment assignments. At the time of calving, fecal, blood and colostrum samples were obtained from each cow and a pre-suckle blood sample was obtained from each calf. Blood samples were

obtained from calves at 6, 12, and 24 hours and at 7, 14 and 21 days post-partum. Serum total

protein and *E. coli* O157:H7 SRP® antibody concentrations were measured in all calves.

*Serology*

**ELISA and Colostrum Assay**

Immulon-2 ELISA plates (Dynatech Laboratories) were coated with *E. coli* O157:H7

SRP® antigen diluted in coating buffer (1.59 g/L $Na_2CO_3$, 2.93 g/L $NaHCO_3$, pH 9.65). One-

hundred (100) μl of the antigen solution was added to each well of the plate and allowed to

incubate overnight at $4^o$C. The coating antigen was then removed from the plate and replaced

with 200μl blocking buffer (10g/L Poly Vinyl Alcohol, 1L 1xPBS). The plates were then

covered and incubated at $37^o$C for 1-2 hours. The blocking buffer was then removed. Serum

from a calf that was hyper-immunized with the *E. coli* O157:H7 SRP® vaccine was used as a

positive control. The samples and control sera were then diluted to 1:100 in blocking buffer.

Test sera were added to the first and 12th well of the plate in duplicate and diluted 4-fold moving

towards the center of the plate so that the last dilution achieved is 1:2400. The plates were then

covered and incubated at $37^o$C for one hour. Plates were then washed three times with 0.05%

Tween-PBS. The conjugate (sheep anti-bovine IgG H&L HRP; The Binding Site, SanDiego,

CA) was then diluted to 1:1600 in the blocking buffer plus 1% sheep sera. One-hundred (100) μl

of the conjugate solution was added to each well, and then the plate was covered and incubated

at $37^o$C for one hour. The plate was then washed three times with 0.05% Tween-PBS. Pre-

warmed, two-component ABTS (Kirkegaard & Perry, Gaithersburg, MD) was combined and 100

μl was added to each well of the plate. The plate was incubated at room temperature until the

optical density of the positive control wells was between 0.8 and 1.2. Plates were then placed

into the plate reader (BioTek ELx405; Winooski, VT) and read at 405/490nm and mixed prior to

reading. The cut-off value for each series was calculated by multiplying the positive control by 0.5 and plotting it against the sample dilution curves. Sample titers were calculated based on the intersection of the cut-off line and the sample curves. Sample titers were then reported as the reciprocal of the dilution at which the cut-off crosses the sample curve.

### Total Protein

Blood was obtained from the jugular vein of each calf using 9 ml vacuum serum tubes (Greiner Bio-One NA) at each time point. The blood was then allowed to clot while being refrigerated for 12-24 hours. Samples were then centrifuged at 3500 RPM for 10 minutes to achieve serum separation from the clot. Serum was then placed onto the refraction crystal of a commercially available, temperature compensated refractometer (Reichert; Depew, NY). Total protein was read and recorded as g/dL.

### Fecal Culture

Fecal samples were obtained directly from the rectum of each animal as it was restrained at each handling time point. The samples were placed into collection vials and placed on ice. Samples were labeled with sequential numbers to blind treatment assignments. Samples were then sent overnight to the Epitopix LLC testing laboratory. Upon arrival at Epitopix, fecal samples were processed for isolation of *E. coli* O157 by immunomagnetic separation. Samples were weighed and approximately 2 grams of each fecal specimen was placed into a Whirl-pak™ filter bag. Gram-negative broth containing Cefixime (0.05 mg/L), Cefsulodin (10 mg/L) and Vancomycin (8 mg/L) (GNccv) was used to get the fecal specimen in a liquid state for sample processing. Samples were normalized by weight so each sample was present at a ratio of 1 gram feces per 10 mls of GNccv broth. Samples were incubated at 37°C overnight for enrichment of

*E. coli* O157.  Following enrichment, 1 ml of each sample was added to a 96-well plate

containing 20 µl of magnetic Anti-O157 Dynabeads (Invitrogen, Carlsbad, CA).  The enriched

cultures were allowed to incubate with the magnetic beads on a shaker at room temperature for at

least 15 minutes per the manufacturer's directions.  Magnetic particles were recovered and

washed using an 8-channel magnetic PikPen (Bio-Nobile, Turku, Finland).  After the final wash,

the particles were released into 100 µl of wash buffer (PBS containing 0.05% $Tween_{20}$) in a 96-

well plate for plating of *E. coli* O157 that was bound to the magnetic particles.  For plating, 50 µl

was plated onto a Sorbitol MacConkey agar plate containing Cefixime and Tellurite (CT-SMAC)

(Becton Dickinson, Franklin Lakes, NJ) and 50 µl was plated onto a Chromagar-O157 plate

(Chromagar, Paris France).  The inoculum was spread onto each agar plate and the plates were

incubated at 37°C overnight.  The plates were observed for suspect *E. coli* O157 colonies which

were then tested for O157 agglutination using an O157 test kit from Remel (Lenexa, KS) per the

manufacturer's instructions.  Positive samples were sub-cultured to Chromagar-O157 or CT-

SMAC to acquire a pure culture of *E. coli* O157.

*Vaccine Preparation*

The commercially available *E. coli* O157:H7 SRP® vaccine was prepared in the same

manner as reported previously.[6,18,19]  The placebo vaccine contained phosphate-buffered saline

emulsified with the same commercial adjuvant (Emulsigen, MVP Laboratories, Ralston, NE)

used in the vaccine.  Vaccine and placebo bottles were marked A or B to blind the vaccine

administrator to the treatments.  Each cow received 2ml of the corresponding vaccine

subcutaneously in the neck following Beef Quality Assurance guidelines[4].


*Data Analysis*

Data were recorded and summarized using the Excel (Microsoft; Redmond, WA) program.  ELISA results were transformed using a log base 2 function prior to using the data for statistical analysis.  The individual calf served as the experimental unit. Data were analyzed using the wsanova procedure in STATA® (College Station, TX) for repeated measures of an individual.  Cow vaccination treatment, pasture, cow ELISA at the time of calving, calf ELISA, time and their interactions were all presented as possible variables for the model.  Colostrum endpoint ELISA data was analyzed using a generalized linear model using a Gaussian distribution and identity link function.   $P$-values $\leq 0.05$ were considered significant.

### Results and Discussion

None of the cows required assistance during parturition and all calves nursed within 2 hours after birth without assistance.  Vaccination treatment had no effect on the calf serum total protein (TP) level (P > 0.05).  However, the length of time post-birth had a significant effect on calf serum TP levels (P < 0.001) (Figure 3.1).  This is an expected finding given that calves are born agammaglobulinemic until absorption of maternal antibodies from colostrum.[2]  One calf in the study was classified as having failure of passive transfer (TP level < 5.5 g/dL at 24 hours). This resulted in a 5% failure of passive transfer prevalence which would be consistent with other reported prevalence estimates in beef cattle, yet well below that of many dairy estimates.[13,21] The calf which exhibited failure of passive transfer was born to a cow that had very little udder development and milk production at the time of parturition which was likely the main factor for failure of passive transfer.  There was a vaccine treatment by time post-birth interaction observed for the calf serum *E. coli* O157:H7 SRP® antibody levels (P < 0.01, Figure 3.2).  This interaction was explained by no vaccine treatment difference in calf serum *E. coli* O157:H7 SRP® antibody levels pre-suckle but a significant increase in calf *E. coli* O157:H7 SRP post-suckle titers in the

calves born to SRP® vaccinated cows compared to calves born to cows that received the placebo vaccination.

Of the 20 cows enrolled in the study only 19 colostrum samples were collected and analyzed. The average endpoint *E. coli* O157:H7 SRP® antibody titers were different between the two treatments (P < 0.001) at 150 and 16,835 for placebo and vaccinated cows, respectively (Figure 3.3). Natural range assignment was not a significant factor for antibody titer level of either treatment (P > 0.70). This marked difference for antibody titers, between vaccinated and placebo cow's colostrum, is in agreement with earlier work done examining *E. coli* K99 vaccine efficacy and protection in neonatal calves.[1]

Another observation from this study was the length of time post-parturition in which calves achieved adequate passive transfer. The majority of the calves achieved adequate passive transfer (TP = 5.5 g/dL) by 6 hours post-parturition (Figure 3.1). These TP levels were achieved in spite of increased handling of cows and calves immediately after parturition due to experimental conditions. These data suggest that the passive transfer status of neonatal calves may be accurately assessed prior to partial gut closure at 12 hours, and full closure at 24 hours.[21] This may be a useful strategy for producers to employ when operating an aggressive colostrum management program.

All 20 cows were negative for fecal shedding of *E. coli* O157:H7 at all three sampling times. These data do not correspond to previous findings for fecal prevalence of beef cow-calf *E. coli* O157:H7 which reported individual animal level prevalence ranging from 2 to 18% and a herd level prevalence ranging from 87 to 100%.[8,11,15,17] The current study did not employ a true random sample of the herd that would be required to truly establish the herd prevalence. The

focus of the current study was colostrum quality and composition, and *E. coli* O157:H7

prevalence was secondary. Therefore, investigators controlled parity to decrease variation in

colostrum quality between cows of different parity. Selection bias could have been inadvertently

introduced to this study if 4 year old cows are at a lower risk for shedding *E. coli* O157:H7 than

other cows in the herd. Previous studies have reported a greater chance of isolating *E.coli*

O157:H7 through repeated sampling.[17] Some researchers have reported that beef cows may not

detectably shed *E. coli* O157:H7 prior to parturition but will shed this foodborne pathogen within

one week after parturition.[8] The third and last sampling of cows in this study occurred

immediately after parturition and yet no cows were found to be shedding *E. coli* O157:H7. The

exact onset of shedding and the factors related to the induction of shedding warrant further

investigation.

This study documented the speed at which passive transfer can occur in beef cattle

following parturition. Calves were observed to have successful passive transfer in the first 6

hours after birth. This knowledge could be used by producers in situations where the colostrum

intake is unknown or the calf is of high value. A producer or veterinarian could identify passive

transfer failures prior to significant gut closure and intervene with colostrum support. This is

also the first report of successful *E. coli* O157:H7 SRP® antibody passive transfer in beef calves

under natural range conditions. These data provide information for further study into possible

cross protection of this vaccine against neonatal *E. coli* diarrhea strains such as K99. This

research is the first step of understanding lifecycle immunization strategies against *E. coli*

O157:H7 in cattle and its effects on shedding of the organism by the animal at the point of

harvest.

**Endnotes**

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Figure 3.1: Average total protein of calves in the vaccination (squares) and placebo (diamonds) treatment groups at pre-suckle, and 6, 12, 24 hours post-suckle. Error bars signify the standard deviation for the measurements at the given time point.
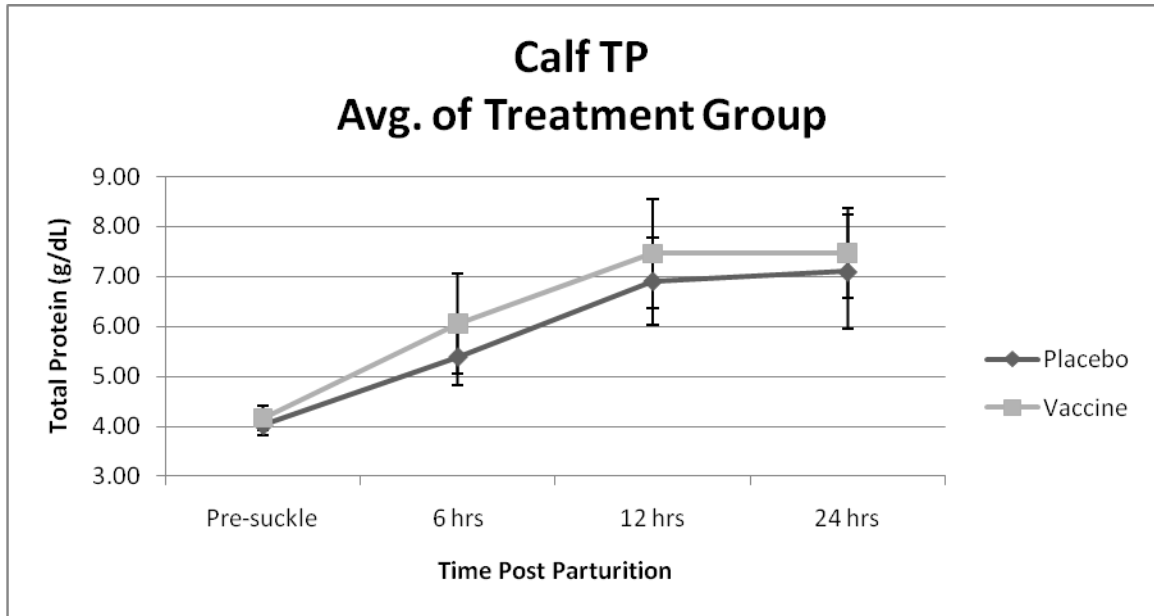
Figure 3.2:  Average *E. coli* O157:H7 SRP® antibody of calves in the vaccination (squares) and placebo (diamonds) treatment groups at pre-suckle 6, 12, and 24 hrs and 7, 14 and 21 days post-suckle.  Error bars signify the standard deviation for the measurements at the given time point.
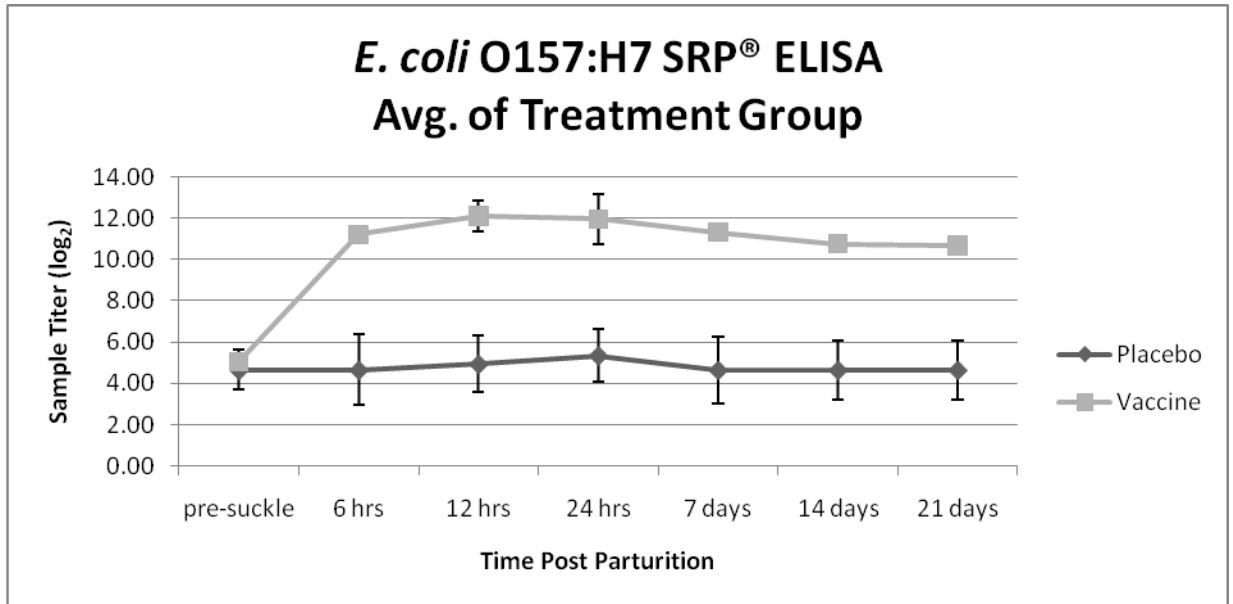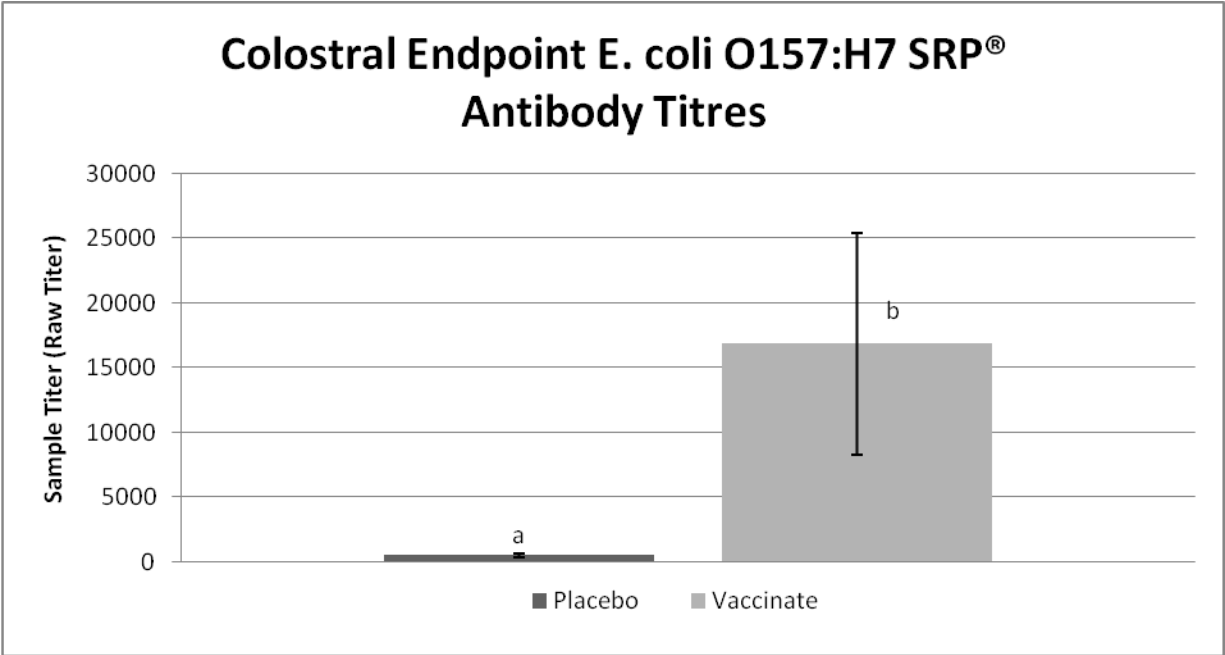
Figure 3.3: Average endpoint *E. coli* O157:H7 SRP® antibody titers of colostral samples by vaccination and placebo treatment groups. Error bars represent the standard deviation.

# CHAPTER 4 - Passive Immunity of Neonatal Calves Given Colostrum Containing *Escherichia coli* O157:H7 SRP Antibodies to an *Escherichia coli* K99 Challenge

## Introduction

Neonatal calf diarrhea is one of the leading causes of morbidity and mortality in calves (Combs et al. 1993). The most commonly described causative agents of acute neonatal diarrhea (scours) are *Escherichia coli*, coronavirus, rotavirus, and *Cryptosporidium*. *E. coli* strains that are K99 positive have been responsible for an estimated 25-30% of the scours cases in calves in the first 3-4 days after birth (Acres 1985; Holland 1990; Naylor 2002). *E. coli* K99 positive organisms have been responsible for 30-50% of the scours-related deaths in neonatal calves (Acres 1985; Combs et al. 1993).

Calves are born agammaglobulinemic due to the separation of fetal and maternal blood flow that prevents the transfer of immunoglobulins from cow to fetus in utero (Arthur et al. 1996). As a result the calf is reliant upon the immunoglobulins contained in the colostrum for its protection from disease challenge in the first 2-4 weeks of life (Barrington & Parrish 2001). Therefore, passively derived antibodies from colostrum are an important component in the calf's ability to defend itself from pathogens. Colostrum feeding management protocols have been reported as a leading cause of mortality in the first 21 days of a calf's life (Wells et al. 1996).

The ability to protect a calf through colostrum derived antibodies has been studied for many years. Protection afforded to neonatal calves by colostrum containing *E. coli* K99 specific antibodies has been previously reported (Combs et al. 1993; Acres et al. 1979; Eichhorn et al. 1982; Gouet et al. 1978; Nagy 1980; Riedel-Caspari 1993; Snodgrass et al. 1982; Valente et al. 1988). These studies have illustrated the wide range of morbidity and mortality outcomes

possible with *E. coli* K99 challenge in both controlled and field conditions.  Morbidity and mortality estimates, across studies, range from 0-100% with an average morbidity and mortality rate of 63% and 33%, respectively.

All of these studies used *E. coli* K99 specific colostral antibodies derived from vaccinating the cows with an *E. coli* K99 derived vaccine.  Recently an *E. coli* O157:H7 SRP® vaccine for cattle was granted licensure by the USDA.  The unique mechanism of action of this vaccine makes it a candidate for further study of uses in the cattle industries.  The efficacy of this vaccine has been demonstrated to reduce fecal shedding of *E. coli* O157:H7 under experimental and field conditions (Fox et al 2009; Thomson et al. 2009; Thornton et al. 2009) and also passive immunity under field conditions (Wileman et al. 2010).  To date there have been no studies reported that have examined the possibility of cross protection of *E. coli* O157:H7 against *E. coli* K99 challenge.  The goal of this study is to examine the degree of cross protection provided by colostrum containing a known amount of *E. coli* O157:H7 antibodies against an oral challenge of *E. coli* K99 as evidenced by morbidity effects in neonatal colostrum deprived calves.

## Methods & Materials

### *General Overview*

This protocol and project was approved by the Kansas State University Institutional Animal Care and Use Committee.  Eleven neonatal, colostrum deprived male Holstein calves were obtained from a local dairy calving facility and transported to a climate controlled research facility in Willmar, MN.  Prior to their procurement, calves were randomly assigned to one of two treatment groups: 1) colostrum from non-vaccinated heifers or 2) colostrum from heifers

vaccinated with SRP *E. coli* O157:H7 vaccine prior to calving. Each calf was uniquely

identified via individually numbered ear tags placed in the ear at the time of arrival to the

research facility. Calves were individually housed on sawdust bedding with a separate footbath

assigned to each pen to prevent cross contamination during husbandry and scoring procedures.

Calves were administered the *E. coli* challenge strain orally one hour after colostrum treatment

administration. Calves were observed twice a day (day 0-7) and a hydration (0 or 1), fecal (1-4),

respiratory (1-3) and an appetite score (1-3) was recorded. Upon completion of the study all

surviving calves were maintained at the research facility until all clinical signs of diarrhea were

resolved with no recurrence for 72 hours and then were sold to a commercial calf raiser.

### *Colostrum Preparation*

Prior to initiation of the study, dairy heifers were vaccinated three times with *E.coli*

O157:H7 SRP® at 90, 60 and 30 days prior to parturition and the colostrum from these heifers

was collected. The colostrum was pooled and placed into 3 quart aliquots and labeled (SRP

colostrum). Colostrum was also collected from non-vaccinated heifers, pooled and placed in 3

quart aliquots (control colostrum). For blinding study personnel, the final bags of colostrum that

were administered to the calves were labeled A or B, corresponding to dam vaccination status.

Calves were administered the colostrum via esophageal tube one hour prior to challenge.

### *Observation and Sampling*

Each calf was assessed and a score was recorded for hydration, fecal consistency, rectal

temperature and appetite upon arrival to the research facility, one hour post-challenge and twice

daily thereafter. All animal caretakers and scoring individuals were blinded as to the assigned

treatment group of each calf. Blood was collected prior to colostrum administration and then

every 24 hours thereafter until completion of the study (day 7) for measurement of *E. coli* O157:H7 specific SRP antibody to monitor passive transfer of maternal antibodies. Hydration was assessed on all calves daily via visual observation and a skin tent test. A binomial hydration score of 0 was given for normally hydrated calves, eyes bright with pliable skin or 1 having dehydration, eyes dull or sunken and loss of skin pliability. Fecal consistency was scored twice daily with the following scores: 1 (normal consistency, firm & well formed), 2 (mild diarrhea, softer than normal but not runny), 3 (moderate diarrhea, runny but some texture), and 4 (having severe diarrhea where the feces was very runny with very little texture). Rectal temperatures were taken via digital thermometer and recorded twice daily. Appetite was scored on a three point scale with 1 indicating a normal appetite, 2 indicating a slightly depressed appetite and 3 indicated inappetence. And respiration was scored by 1 (normal respiratory effort), 2 (mild increase in respiratory effort) and 3 (labored respiratory effort). An approximately 2 gram fresh, fecal sample was obtained on arrival to the research facility and then once daily for culture of the *E. coli* K99+ challenge organism.

### *Challenge Strain*

Five hundred ml of pre-warmed (37$^{o}$C) Tryptic Soy Broth (TSB) (Becton Dickinson, Franklin Lakes, NJ) containing 20μg/ml of dipyridyl (Sigma, St. Louis, MO) and 100μg/ml of nalidixic acid (Sigma, St. Louis MO) were inoculated with 5μl of K99 *E. coli* strain 1126. The bottle was incubated on a shaker at 37$^{o}$C for 13.5 hours. Twenty-Five (25) ml of the culture were then passed into eight bottles containing ~1,350 ml of the same media. The bottles were then incubated on a shaker at 37$^{o}$C until an OD$_{545}$ was between 1.1 and 1.4 (4 hours). The culture was then harvested by centrifugation using a Beckman JA-10 rotor at 6,000 RPM for 20 minutes. The supernatant was discarded and the pellets were re-suspended until a 1:1,000

90

dilution in TSB yielded an $OD_{545}$ of 0.3. Ten ml of this suspension were added to 35 ml of saline which served as 1 calf dose and was given to each calf via an esophageal tube. The titer of the culture was determined by performing 10-fold dilutions of the culture in saline, plating the dilutions on blood agar plates, incubating and counting colonies. The titer was checked for each challenge preparation before and after challenge. The titer of the challenge dose per calf was 1 x $10^{11}$ CFU of *E. coli* K99.

### *Serology*

Serum antibody response was determined by enzyme-linked immunosorbant assay (ELISA). *E. coli* O157 SRP antigen was coated on Nunc Maxisorp 96 well plate (Nalge Nunc International  Rochester, NY) at 250ng/well in Carbonate Coating Buffer (pH 9.6), covered and incubated overnight at 4°C. Plates were then dumped and blocked using 1% PVA/PBS, covered and incubated at 37°C for one hour. Dilutions of the serum samples were prepared in 1% PVA/PBS at 1:100 and then serially four-fold diluted. Plates were then covered and incubated at 37°C for one hour. The plate was then washed three times with 0.05% PBS-Tween 20. Following the wash step a 1:1600 dilution of the conjugate, HRP sheep anti-bovine IgG (The Binding Site  San Diego, CA), in 1% PVA/PBS was applied to the plate, covered, and incubated at 37°C for one hour. The wash step from above was repeated prior to development with  2,2' azino-di-3-ethyl-benzthiazoline-6-sulfonate (ABTS) (KPL, Inc.  Gaithersburg, MD). The absorbance of the wells was then read at 405-490nm using an ELISA reader. Using the optical density values of the Positive control, a cut-off line was calculated by multiplying the mean of the positive control by 50%. The optical density and dilution data of the samples was then graphed and the cut-off line was applied. The sample titers were calculated based on where the

cut-off line (PC) intersected the sample curves.  The sample titer was reported as the reciprocal of the dilution at which the cut-off (PC) crosses the sample curve.

### *E. coli K99 Culture*

The presence and concentration of the *E. coli* K99 challenge strain was determined by plating the fecal samples on Eosin Methylene-Blue (EMB) agar (EMD  Gibbstown, NJ) containing 100μg/ml Nalidixic acid (Sigma  St. Louis, MO).  Briefly, for each sample, approximately 2 grams of fecal material was mixed with 20ml Tryptic Soy  Broth (BD  Sparks, MD) with 100μg/ml Nalidixic acid in a filter bag.  One milliliter of the filtrate was removed and diluted out to $10^{-5}$ and plated.  The remaining fecal suspension was incubated at 37°C overnight, and then plated.  The Nalidixic acid resistant challenge strain colonies present on the titration plates were counted, and the enrichment plates were documented as positive or negative for growth of the challenge strain.

### *Data Analysis*

All observations and clinical scores were recorded on a standard observation sheet for each calf.  Scores were transcribed from the written forms to an Excel® spreadsheet for data manipulation and reporting of descriptive statistics.  Data were statistically analyzed in STATA® using linear regression and ordered logistic regression models with treatment, day of study and their interactions submitted as predictors.

## Results

A total of five calves died prior to study termination from causes unrelated (clinical signs or death prior to administration of challenge dose) to *E. coli* K99 challenge (3 from SRP group, 2

from control group). Colostrum treatment assignment had no significant effect on rectal temperature, attitude, respiratory, or hydration scores of calves ($P > 0.05$). Day of challenge was significantly predictive of rectal temperature, attitude, respiratory, and hydration scores of calves ($P < 0.05$). There were no significant day by colostrum treatment interactions for any of the parameters ($P > 0.05$). SRP colostrum treated calves had significantly improved fecal scores compared to control calves ($P = 0.05$; Figure 4.1). Colostrum treatment had no effect on elimination of fecal shedding of *E. coli* K99 by calves ($P > 0.05$). However, SRP colostrum treated calves had a 0.45 log CFU/ml lower concentration of *E. coli* K99 in feces ($P = 0.05$; Figure 4.2). Control colostrum calves had significantly lower serum *E. coli* O157:H7 SRP antibodies than SRP colostrum treated calves ($P < 0.001$; Figure 4.3).

## Discussion

The death loss incurred at the beginning of the study severely limited the statistical power of this trial to detect differences between treatment groups. The use of an *E. coli* O157:H7 vaccine pre-partum has largely been unstudied as to its ability to protect the calf from *E. coli* O157 infection/colonization and its ability to cross-protect against other pathogenic species such as K99. This is the first report using this approach. In spite of very limited statistical power there does appear to be encouraging results in a significant improvement in fecal consistency (Figure 4.1), reduction in concentration of shedding of challenge organism (Figure 4.2) and a strong antibody response (Figure 4.3).

The experimental efficacy of vaccines administered to cows prior to calving and the subsequent protection of the calf post-colostral intake has been reported in the literature extensively (Jayappa et al. 2008; Crouch et al. 2001; Combs et al. 1993; Eichhorn et al. 1982). However few studies go into the field and examine the "field efficacy" of this management

procedure while controlling for other management issues. In a large observational study Trotz-Williams et al. (2007) found a significant reduction in the likelihood (OR 0.58 95% CI 0.41-0.81) of a calf being diagnosed with diarrhea when it was born to a dam previously vaccinated with a vaccine containing *E. coli* antigens. In that study enterotoxigenic *E. coli* isolates were present in 51% of all of the fecal samples. Interestingly, they also reported a significantly increased likelihood of diarrhea in calves born during the summer rather than the winter which is in contrast to other reports (Waltner-Toews et al. 1986; Frank and Kaneene 1993). The current study was conducted in Minnesota during the month of January. Although the calves were housed in controlled conditions during the study, prior to their procurement they were born and retrieved from maternity pens in hoop-barns, placed into a warm calf shed, and then transported one hour to the research facility. In the author's opinion the early morbidity and mortality that was unrelated to the trial was largely due to affects of hypoxia/hypothermia as all calves were necropsied with no lesions found indicating disease or calving trauma (data not shown) and clinical signs (if present) were present prior to challenge dose administration.

*Escherichia coli* O157:H7 from which the vaccine used in the current trial was created has been shown to be virulent to neonatal calves capable of producing morbidity and even mortality (Dean-Nystrom et al. 1997). In a field study, calves with clinical diarrhea that were two weeks old or younger had an *E. coli* O157:H7 prevalence of 15.8% whereas the clinically normal calves had 0% prevalence (Kang et al. 2004). This was a small power study performed in South Korea so the production practices related to the U.S. is unknown, but does allude to *E. coli* O157:H7 as being a possible cause of neonatal diarrhea. The current study did not attempt to examine the fecal samples for *E. coli* O157 as the K99 isolates are much more common in reported field isolates (Holland 1990; Naylor 2002; Trotz-Williams et al. 2007).

This trial confirms the findings of Wileman et al. (2010) of successful passive transfer of *E. coli* O157:H7 SRP specific antibodies in calves. The field trial used beef breed calves housed under natural range conditions whereas the present trial utilized dairy breed animals administered colostrum via an esophageal tube feeder. In a recent study Godden et al (2009) reported an interaction between the amount of colostrum fed (1.5L vs 3.0L) and the method it was delivered (esophageal feeder vs nipple) on serum total protein (TP) and IgG concentrations. There was no difference in serum TP or IgG between calves fed 3.0 L of colostrum with a nipple or an esophageal feeder. However, calves fed 1.5 L of colostrum via nipple had improved apparent efficiency of absorption of IgG over calves fed via esophageal tube. In the present study the calves were administered approximately 2.8 L of colostrum via esophageal tube and the resulting serum ELISA titers of antibodies to *E. coli* O157:H7 SRP group calves were very similar to those reported by Wileman et al (2010) for beef calves consuming colostrum under natural conditions.

Due to the encouraging results, further study is warranted into the use of the *E. coli* O157:H7 SRP vaccine pre-partum through the refinement and repetition of this trial during a warmer period of the year with stricter enrollment criteria and additional isolation procedures for *E. coli* O157:H7 organisms in fecal samples. It does appear that vaccination of cows pre-partum with *E. coli* O157:H7 SRP vaccine may provide additional benefits against clinical signs and shedding of pathogenic *E. coli* K99 in calves through passive immunity. Decreased shedding of *E. coli* K99 into the immediate neonatal environment would decrease the likelihood of large neonatal diarrhea outbreaks in intensive systems. Further study into the cross-protective ability of the *E. coli* O157:H7 SRP vaccine against K99 and other pathogenic strains of *E. coli* is needed.

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**Figure 4.1:** Average fecal consistency score of calves (1=Normal, 2= Mild, 3=Moderate, 4=Severe) by day of observation.  SRP colostrum had improved fecal consistency scores compared to control calves (P = 0.05).
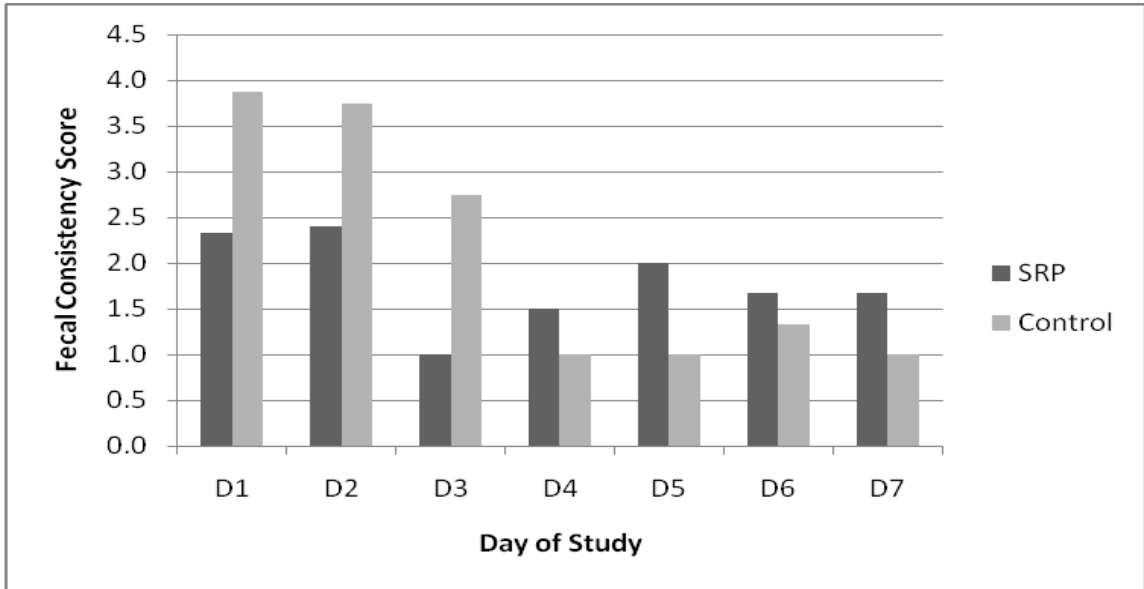
**Figure 4.2:** Fecal *E. coli* K99 concentration ($\log_{10}$ CFU/ml) by day of study. SRP colostrum calves had lower concentrations of E. coli K99 shed in feces compared to control calves (P = 0.05).
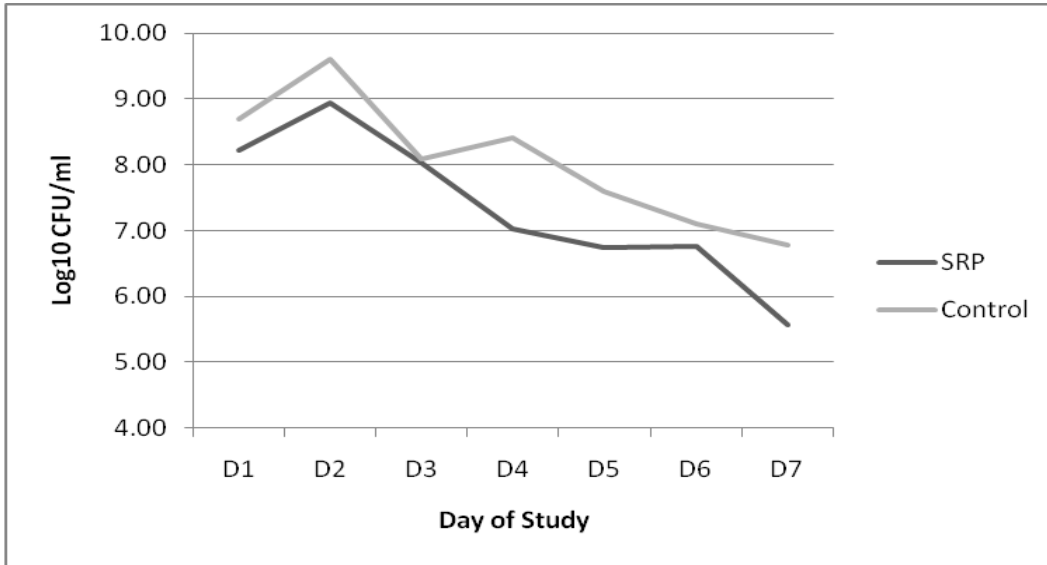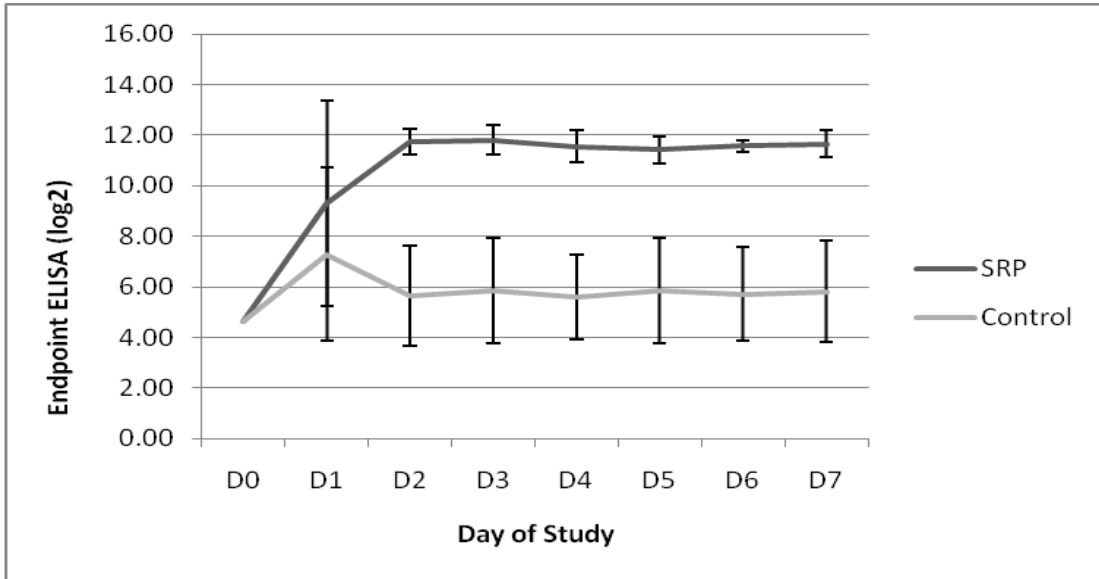
**Figure 4.3**: Serum Anti-*E. coli* O157:H7 SRP Antibody (log2) by day of study. Error bars represent the standard deviation of the titer at each time point.  SRP colostrum calves had increased anti-E. coli O157:H7 titers compared to control calves ($P < 0.001$).

# CHAPTER 5 - *Escherichia coli* O157:H7 Shedding in Beef Calves Vaccinated with *Escherichia coli* O157:H7 SRP® Vaccine Born to Cows Vaccinated Pre-partum with *Escherichia coli* O157:H7 SRP® Vaccine

## Introduction

Enterohemorrhagic *Escherchia coli* 0157:H7 is one of the most recognized human, foodborne pathogens in the United States and around the world. *E. coli* was first recognized as a human foodborne pathogen in the early 1980's after two outbreaks linked to undercooked hamburgers (Riley et al. 1983). There are three manifestations of infections in humans: hemorrhagic colitis, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Griffin & Tauxe 1991). Signs of human infection occur 3-5 days after ingestion of the organism. Most cases are self limiting in that only 5% of cases result in the severe complications of HUS or TTP (Bach et al. 2002). The groups of people most at risk of developing the severe complications are children under the age of 5 years old, the elderly and immunocompromised individuals. The largest numbers of reported cases are in the countries of the United States, Canada and the United Kingdom (Griffin & Tauxe 1991). The 2006 reported incidence of *E. coli* 0157 infections in the U.S. by the Center for Disease Control's FoodNet was 1.31 per 100,000 which is half of what the incidence was just 10 years prior. Much of the decrease is likely related to peri- and postharvest intervention strategies implemented through the hazard analysis and critical control points (HACCP) system in all FDA regulated harvest facilities.

A major development in the ecology and epidemiology of *E. coli* O157 was the establishment of cattle as the reservoir for *E. coli* O157:H7 (Riley et al. 1983). This spurred a

large amount of research focused specifically on *E.coli* 0157:H7 and its involvement with beef cattle. Early prevalence estimates estimated the herd level prevalence of *E. coli* O157 to be around 8-16% (Bach et al. 2002). The development of more sensitive testing procedures such as immunomagnetic separation (IMS) has increased the sensitivity of detection of the organism by 10-100 fold (Chapman et al. 1994). This has resulted in more accurate and increased estimates of the true prevalence of *E. coli* O157:H7 in the U.S. beef herd. A study by Elder et al. (2000) found a feedlot-level prevalence of 63% and an individual animal-level prevalence of approximately 28%. The prevalence of *E. coli* O157 is affected by season, the testing frequency and timing of sampling and the treatment of the samples prior to isolation, and the geographic location (Bach et al 2002; Rhoades et al. 2009). In the U.S., the summer months of May through September tend to have the highest prevalence estimates of *E. coli* O157:H7 in cattle feces with the winter months having the lowest prevalence (Van Donkersgoed et al. 1999).

There are a number of pre-harvest intervention strategies that have been reported. In two separate reviews of pre-harvest control of *E. coli* O157:H7, the reviewing authors found that the probiotic containing *Lactobacillus acidophilus* NP51 and *P. freudenreichii* was the only intervention strategy that has been significantly and consistently proven in field trials to increase animal resistance to infection with *E. coli* O157 (Sargeant et al. 2007; Lejune &Wetzel 2007). Sargeant et al. (2007) also concluded that sodium chlorate in the feed or water also increased animal resistance to infection with *E. coli* O157 even though it has not been labeled for use in the U.S. Neither of the reviews considered the vaccine technology utilizing *E. coli* O157:H7 SRP[®], for controlling *E. coli* prevalence in cattle pre-harvest. This technology has recently received conditional licensure in the U.S. and has been extensively studied for control of *E. coli* prevalence and concentration in feeder cattle feces (Fox et al. 2009; Thomson et al. 2009;

Thornton et al. 2009; Wileman et al. 2010).  The majority of existing research and control strategies attempt to control *E. coli* O157 at the feedyard and harvest stages of production.  These approaches are addressing the problem post-colonization.  The objectives of this study were to determine the shedding characteristics, health and performance of *E.coli* O157:H7 SRP throughout the lifetime of beef calves born to *E.coli* O157:H7 SRP vaccinated dams verses unvaccinated dams.  Also, this study examined the effects of a series of *E. coli* O157:H7 SRP vaccinations in beef calves prior to and at the time of arrival at the beef finishing facility had on the *E. coli* O157:H7 shedding characteristics, health and performance throughout their lifetime.

## Materials and Methods

### *General Overview*

This study was conducted under conditions approved by the Kansas State University Institutional Animal Care and Use Committee.  In January of 2009, 437 cows from the Kansas State University two commercial cow-calf herds were stratified by age and randomly assigned to either *E. coli* SRP vaccine or placebo treatment groups.  Blood samples were collected from cows prior to the initial vaccination to ensure an *E. coli* O157:H7 SRP[®] antibody free status.  Cows were vaccinated with their assigned vaccine treatments at 60 and 30 days prior to the projected start of the calving season.  To create a 2X2 factorial treatment structure, calves were then blocked by dam treatment and randomly assigned to *E. coli* SRP vaccine or placebo treatment groups which were administered at branding, preconditioning and entry to the feedlot.  Therefore, there were a total of four treatments in this study: 1) neither cows nor calves vaccinated with *E. coli* O157:H7 SRP (CON), 2) cows vaccinated with *E. coli* O157:H7 SRP technology pre-partum but calves not vaccinated (COWVAC), 3) calves vaccinated with *E. coli*

O157:H7 SRP technology born to cows not vaccinated (CALFVAC), 4) cows vaccinated with *E. coli* O157:H7 SRP technology pre-partum and calves also vaccinated (BOTH). The two vaccine treatments were prepared by the sponsoring company and labeled A & B to blind study personnel. Also, all blood and fecal samples were labeled with sequential numbers to blind laboratory personnel.

Cows and calves were managed according to normal ranch management conditions at each unit throughout the study. While on pasture cows & calves from the four treatment groups were allowed to commingle i.e. vaccinates with placebo. During the preconditioning and feedlot phases of the trial, calves were penned according to sex and vaccine treatment group. A 30 day ranch preconditioning trial was also conducted and balanced across the current trial's treatment groups. Calves were gathered while on grass at the traditional branding time age of approximately 90 days old. Calves were given a 7-way *Clostridial* vaccine and their first assigned SRP vaccine treatment, had blood and fecal samples collected, and the males were castrated. Calves were returned to their dams and turned out to grass pastures until weaning. Calves were abruptly weaned and transported to preconditioning pens at each of the two ranches where they were processed, administered the second dose of SRP vaccine and provided ad libitum access to fresh grass hay and water. At the end of the 30 day preconditioning period calves were transported by truck 3 hours to an auction facility where they remained overnight, and then were reloaded and transported to the feedyard. Calves were processed on arrival to the feedyard and administered their third and final SRP vaccine dose if assigned to that treatment. Feedlot pens were created by combining a matched (matched by sex and treatment assignment) preconditioning pen from each of the two ranches. Calves remained in these pens for the remainder of the study. Health and performance data were collected monthly.

## Serology

Serum antibody response was determined by enzyme-linked immunosorbant assay (ELISA). *E. coli* O157 SRP antigen was coated on Nunc Maxisorp 96 well plate (Nalge Nunc International Rochester, NY) at 250ng/well in Carbonate Coating Buffer (pH 9.6), covered and incubated overnight at 4°C. Plates were then dumped and blocked using 1% PVA/PBS, covered and incubated at 37°C for one hour. Dilutions of the serum samples were prepared in 1% PVA/PBS at 1:500 in duplicate. Plates were then covered and incubated at 37°C for one hour. The plate was then washed three times with 0.05% PBS-Tween 20. Following the wash step a 1:1600 dilution of the conjugate, HRP sheep anti-bovine IgG (The Binding Site San Diego, CA), in 1% PVA/PBS was applied to the plate, covered, and incubated at 37°C for one hour. The wash step from above was repeated prior to development with 2,2' azino-di-3-ethyl-benzthiazoline-6-sulfonate (ABTS) (KPL, Inc. Gaithersburg, MD). The absorbance of the wells was then read at 405-490nm using an ELISA reader. The average of the negative controls' optical density was calculated and subtracted from all values as a reagent blank. Then the sample duplicates were averaged and divided by the positive control average yielding the sample to positive (S:P) titer.

## Fecal Culture

Fecal samples were obtained directly from the rectum of each animal as it was restrained at each handling time point. The samples were placed into collection vials and placed on ice. Samples were labeled with sequential numbers to blind treatment assignments and were sent overnight to the Epitopix LLC testing laboratory. Upon arrival at Epitopix, fecal samples were processed for isolation of *E. coli* O157 by immunomagnetic separation. The same procedure previously described by Wileman et al. (2010) was used for the branding, preconditiong and

feedlot arrival samples.  The simulated slaughter fecal samples used the same procedure except

samples underwent a six hour enrichment rather than overnight.  Briefly, samples were weighed

and approximately 2 grams of each fecal specimen was placed into a Whirl-pak™ filter bag.

Gram-negative broth (GNccv) was used to get the fecal specimen in a liquid state for sample

processing.  Samples were normalized by weight so each sample was present at a ratio of 1 gram

feces per 10 mls of GNccv broth.  Samples were incubated at 37°C overnight (6 hours for

simulated slaughter samples) for enrichment of *E. coli* O157.  Following enrichment, 1 ml of

each sample was added to a 96-well plate containing 20 µl of magnetic Anti-O157 Dynabeads

(Invitrogen, Carlsbad, CA).  The enriched cultures were allowed to incubate with the magnetic

beads on a shaker at room temperature for at least 15 minutes.  Magnetic particles were

recovered and washed using an 8-channel magnetic PikPen (Bio-Nobile, Turku, Finland).  After

the final wash, the particles were released into 100 µl of wash buffer (PBS containing 0.05%

Tween$_{20}$) in a 96-well plate for plating of *E. coli* O157 that was bound to the magnetic particles.

For plating, 50 µl was plated onto a Sorbitol MacConkey agar plate containing Cefixime and

Tellurite (CT-SMAC) (Becton Dickinson, Franklin Lakes, NJ) and 50 µl was plated onto a

Chromagar-O157 plate (Chromagar, Paris France).  The inoculum was spread onto each agar

plate and the plates were incubated at 37°C overnight.  The plates were observed for suspect *E.

coli* O157 colonies which were then tested for O157 agglutination using an O157 test kit from

Remel (Lenexa, KS).  Positive samples were sub-cultured to Chromagar-O157 or CT-SMAC to

acquire a pure culture of *E. coli* O157.

## *Data Analysis*

Data were recorded and summarized using the Excel (Microsoft; Redmond, WA)

program.  For feed efficiency (FE) and dry matter intake (DMI) the pen was the experimental

unit, for all other factors the calf was the experimental unit. The majority of the heifers enrolled in this study were placed onto a heifer development trial therefore only data from steers and cull heifers destined for slaughter (n = 252) were used for the reported feedlot entry and slaughter statistics. Data were statistically analyzed using GLIMMIX and Mixed procedures in SAS$^{®}$ 9.1 (SAS Institute Inc.; Cary , NC). Lung lesions, liver abscess, morbidity and mortality data was given a binomial score, summarized and modeled using an events/trial format with feedlot pen as a random effect using the GLIMMIX procedure. Model adjusted risk probabilities were then calculated for these outcomes. Cow vaccination, calf vaccination, ranch of origin, preconditioning trial treatment and their interactions were presented as possible variables in each model. P-values ≤ 0.05 were considered significant.

## Results

The final number of cattle in each treatment group was 105 CON, 103 CALFVAC, 100 COWVAC and 108 BOTH for a total of 416 head. None of the calves were found to be shedding *E. coli* O157 on fecal cultures at the time of initial vaccination at branding. Calves born to vaccinated cows had significantly increased titers of anti-*E. coli* O157:H7 SRP antibodies (SRPAb) in circulation at branding (P < 0.001). Only three calves were shedding *E. coli* O157 at weaning, and all three were placebo calves. At weaning calves from ranch number one had significantly greater SRPAb titers than did calves from ranch two (P = 0.009). All four treatment groups, CON, CALFVAC, COWVAC and BOTH, had significantly different titers (P < 0.001) from each other during the feedlot phase with the CALFVAC calves having the highest average titer followed by BOTH calves (Figure 5.1). Due to the nature of the study design, when a longitudinal data analysis of SRPAb was performed accounting for the repeated samplings of

the same animal over time, a significant three-way interaction between time, cow treatment and calf treatment was identified (P = 0.008; Figure 5.1).

Upon feedlot entry there was an overall fecal *E. coli* O157:H7 prevalence of 34% among calves. The fecal *E. coli* O157 prevalence at entry was CON (25%), CALFVAC (19%), COWVAC (32%) and BOTH (15%) which were not significantly different from one another (P > 0.05; Figure 2). *E. coli* O157 fecal prevalence based on just the calf vaccination treatment was vaccinate (17%) and placebo (31%). At slaughter, overall fecal *E. coli* O157 prevalence decreased slightly to 27%. Fecal *E. coli* O157 prevalence at slaughter was CON (22%), CALFVAC (32%), COWVAC (15%) and BOTH (39%) which were not significantly different from one another (P > 0.05; Figure 5.2). *E. coli* O157 fecal prevalence based on just the calf vaccination treatment was vaccinate (36%) and placebo (17%). There was an unequal distribution of shedding prevalence between pens. Three of the eight pens had fecal *E. coli* O157 prevalence greater than 45%, two vaccinate pens and 1 placebo pen. One pen had a prevalence of 13% while the remaining four had a prevalence of 4% or less. Seventeen percent (11/65) of the calves shedding *E. coli* O157 at slaughter were also shedding at feedlot entry. Nearly all (10/11) of these calves were from one of the three very high prevalence pens (Figure 5.3). Fecal shedding of *E. coli* O157 on arrival to the feedlot was not correlated to fecal shedding at slaughter (Spearman's Rho = -0.02; P = 0.91). There were no significant effects of cow or calf *E. coli* O157:H7 SRP vaccination treatment on feedlot health or performance of calves (P > 0.05; Table 5.1). There were also no vaccination effects on the prevalence of lung lesions or liver abscess (P > 0.05; Table 5.1), nor the number of morbidities, re-treatments or mortalities (P > 0.05; Table 5.1).

## Discussion

Nearly all of the previously reported studies for pre-harvest *E. coli* O157 control have been performed on cattle in the feedlot (reviewed in Lejune & Wetzel 2007; Sargeant et al. 2007; Loneragan & Brashears 2005). This is the first trial to study the effects of vaccination of the dam in order to prevent colonization of the calf pre-harvest, through passive transfer, using the SRP vaccine technology. Our laboratory had previously reported the successful passive transfer of *E. coli* O157:H7 SRP specific antibodies using a subsample of calves from the current study (Wileman et al. 2010). Protection of neonates from disease through manipulation of colostral components via vaccination of the dam pre-partum is a long studied method for pathogenic viral and bacterial diseases (Acres et al. 1979; Fulton et al. 2004; vanDrunen et al. 2008). However, protection of calves against a relatively non-pathogenic commensal organism through passive immunization is much less studied. A small study done in Japan also showed successful passive transfer of *E. coli* O157 antibodies to calves but did not follow the calves to report whether this was protective (Widiashi et al. 2004). Interestingly, a study in Argentina examining the ability of bovine colostrum-derived antibody at inhibiting hemolytic activity of attaching and effacing *E. coli* found that colostral lactoferrin was also very important in inhibition of hemolytic activity (Vilte et al. 2008). Lactoferrin is an iron-binding glycoprotein in colostrum and milk that has inflammatory, antimicrobial and immunomodulatory functions (Brock 2002). Thus lactoferrin and the SRP based technology are combating the *E. coli* organism by using its need for iron against itself (Neilands 1995). It would be assumed that the combination approach of antibodies against the iron acquisition proteins and lactoferrin sequestering free-iron stores that colonization of *E. coli* O157 early in the life of cattle would be additively inhibited. However throughout the

feedlot phase of the current study, fecal *E. coli* O157 prevalence remained at elevated levels through harvest.

There was a significant increase in the prevalence of *E. coli* O157 from the time of weaning (0.6% prevalence) until the time of harvest (27% prevalence). What caused the increase is not known but it does mirror other reports where prevalence of *E. coli* O157 was low (0-1.9%) in calves while on pasture (Gannon et al. 2002; Riley et al. 2003; Sargeant et al. 2000). In a similar longitudinal trial Gannon et al. (2002) demonstrated the apparently cyclic nature of *E. coli* shedding as between 1-7 weeks of age fecal prevalence was 25%, then after turning out to pasture the prevalence went to 0% only to return 6-14% by two weeks post-weaning. The current study found a similarly increasing prevalence with the branding time (already on grass) prevalence of 0%, weaning prevalence of 0.6%, feedlot entry prevalence of 34% and a harvest prevalence of 27%. Unlike the Gannon study, the current study did not sample calves prior to 7 weeks of age but did follow the calves through until harvest. The prevalence estimates reported for the feedlot and harvest phases of this study are within many of the previously reported ranges of feedlot prevalence (Renter & Sargeant 2002; Lejune et al. 2004; Elder et al. 2000; Smith et al. 2001; Chapman et al. 1997).

The pen-level fecal *E. coli* O157 prevalence at feedlot arrival was not correlated with fecal prevalence at harvest. Figure 3 illustrates the range of fecal prevalence observed in this trial with 3 pens (1 placebo & 2 vaccinate) with a very high prevalence compared to the other pens. The interesting finding was that 10 of the 11 calves that were shedding *E. coli* O157 at entry to the feedyard and at harvest were from these three pens. It is possible that these 11 calves are super-shedders and are largely responsible for the increased prevalence in these pens, but unfortunately quantitative culture was not performed on fecal samples in this trial to validate

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this.  This could explain the large increases in shedding in these three pens from entry to harvest as well as finding these animals shedding *E. coli* O157 on two occasions 168 days apart. Previous studies have found that the amount of fecal shedding and the duration of fecal shedding of *E. coli* O157 are increased in super-shedders and is likely a significant contributor to between animal transmission (Naylor et al. 2003; Omisakin et al. 2003; Matthews et al. 2006).  Therefore the possibility of too large of, and persistent of a challenge in these pens may have resulted in the lack of difference between placebo and vaccinate groups.  However, Fox et al. (2009) showed that the SRP vaccine given three times was effective in stopping super-shedders.  The three doses administered in that trial were all given during the feedyard phase.  The results from this trial combined with those of Fox et al. would suggest that vaccination should occur during the feedyard phase of production.

In this trial the *E. coli* O157:H7 specific antibody titers peaked shortly after feedlot arrival and were lower at the time of harvest (Figure 5.1).  Although beyond the scope of this study, it is possible that the serum titer levels dropped below the level of protection that would have resulted in a statistically significant difference in fecal shedding between vaccinates and placebos.  The previously reported studies (Thomson et al. 2009; Fox et al. 2009) using the same vaccine all had the final booster-vaccination administered to yearling cattle within 60 days of harvest and all vaccinations administered within 100 days of harvest.  In the current trial the first vaccination was given at approximately 75 days of age with the second vaccination occurring at 187 days of age and the final vaccination at 217 days of age.  This protocol results in 112 days between the first vaccination and the second and 168 days from final vaccination until harvest. This approach was selected to reflect industry applicable handling time points for administration of the SRP vaccine due to its three dose label.  The ability to spread the three doses over normal

handling time points would be much more preferred than adding an additional handling time during the feedlot phase of the production cycle when performance of the cattle is critical. When the previously reported studies results and intervals from final vaccination to harvest are compared to the current study's results and interval from final vaccination to harvest, it indicates that the timing of the final vaccination relative to the point of harvest could be very important.

The enrichment step in the fecal culture protocol was changed for only the harvest time point samples to a six hour enrichment rather than a 24 hour enrichment. This decision was made to aid the laboratory personnel in identification of suspect colonies based on a personal conversation with researchers at the U.S. Meat Animal Research Center (MARC) about a previous study performed there (Barkocy-Gallagher et al. 2002). The study reported significantly improved sensitivity using the method developed at the MARC due to less enrichment time for competing commensal organisms resulting in less commensal growth on selective media and greater recovery rates of injured cells. The extent to which this affected the results of this trial is unknown as samples were not split and tested in parallel with the two incubation times. In theory the sensitivity should be increased equally across treatment groups resulting in an equal net difference between them. However Barkocy-Gallagher et al. did not discuss whether this affected the lower detectable limit of the procedure. If this did occur than the samples that would have been undetectable with the previous procedure would now become detectable with the current procedure changing a negative sample into a positive sample. The SRP vaccine has been shown to decrease the amount of *E. coli* O157 shed in the feces of cattle (Thomson et al. 2009), thus it is plausible that this decrease could have resulted in a greater number of negative samples, under the longer enrichment procedure in the feedlot entry samples

and a greater number of positive samples, under the shorter enrichment procedure in the harvest samples. Whether this is true and to what extent is unknown.

There were no adverse performance or health effects identified in this study due to *E. coli* O157:H7 SRP vaccination. There was no difference between SRP vaccinated cattle and placebo with regards to fecal *E. coli* O157 prevalence upon entry to the feedyard and at harvest using a modified three dose regimen. However, SRP vaccination did result in a significant increase in serum antibody titers compared to placebo. Further study into the different industry applicable handling time points where the vaccine is likely to be administered is warranted as it appears that timing of vaccination near slaughter may be important. SRP vaccination is an effective strategy for the reduction of fecal shedding of *E. coli* O157:H7 in feedlot cattle. The timing of when this vaccination is applied appears to be a major determinant of its efficacy at reduction in fecal *E. coli* O157:H7 at the time of harvest. *E. coli* O157:H7 SRP vaccine technology is only one part of a comprehensive food safety program that when implemented appropriately will help maintain beef as a safe and nutritious food source.

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**Table 5.1:** Summary of feedlot performance and carcass effects by calf vaccination treatment. Vaccinate or placebo indicates the calf vaccine treatment only, no cow vaccine treatments were found to be significant (P > 0.10). Standard error of the mean (SEM).

| Parameter | Vaccinate | Placebo | SEM | P-value |
|---|---|---|---|---|
| Wean weight (lbs) | 488 | 487 | 15 | 0.96 |
| Feedlot entry weight (lbs) | 528 | 527 | 15 | 0.99 |
| Final bodyweight (lbs) | 1128 | 1134 | 10 | 0.67 |
| Feedlot ADG (lbs/d) | 3.06 | 2.91 | 0.16 | 0.50 |
| Weaning thru slaughter ADG (lbs/d) | 2.79 | 2.65 | 0.15 | 0.50 |
| HCW (lbs) | 750 | 757 | 13 | 0.70 |
| Marbling | 44.47 | 43.85 | 0.90 | 0.64 |
| Back fat (inches) | 0.44 | 0.42 | 0.025 | 0.70 |
| REA (inches) | 12.67 | 12.74 | 0.12 | 0.70 |
| KPH (%) | 2.96 | 3.15 | 0.26 | 0.61 |
| YG | 2.97 | 2.99 | 0.13 | 0.93 |
| DMI (lbs/day) | 22.48 | 22.59 | 0.03 | 0.06 |
| F:G | 6.35 | 6.51 | 0.14 | 0.44 |
| Liver Abscess (Risk) | 0.28 | 0.26 | -- | 0.87 |
| Lung Lesion (Risk) | 0.37 | 0.42 | -- | 0.65 |
| First Pull (Risk) | 0.12 | 0.09 | -- | 0.51 |
| Re-treatment (Risk) | 0.01 | 0.01 | -- | 0.98 |
| Mortality (Risk) | 0.03 | 0.01 | -- | 0.42 |

**Figure 5.1:** Differences in serum anti-*E. coli* O157:H7 SRP antibody by cow treatment and calf treatment at each sampling time point. The study design and the corresponding immunological response of the calves resulted in a three-way interaction between cow treatment*calf treatment*time (P = 0.008).
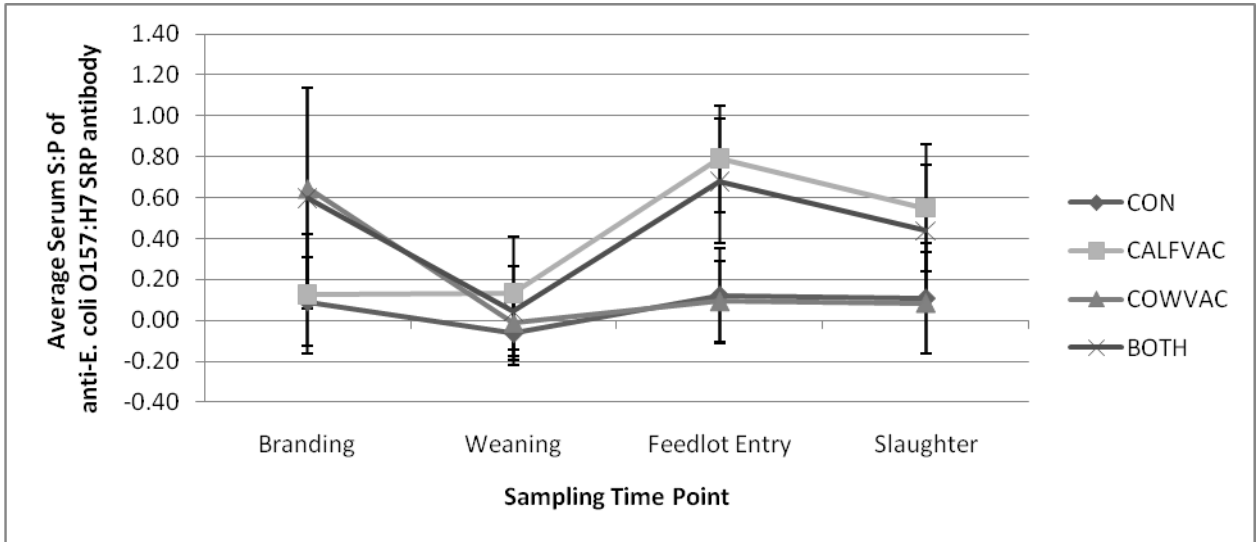
**Figure 5.2:** Fecal *E. coli* O157:H7 prevalence by treatment group at feedlot entry and at slaughter. There were no differences between treatment groups (P > 0.05).

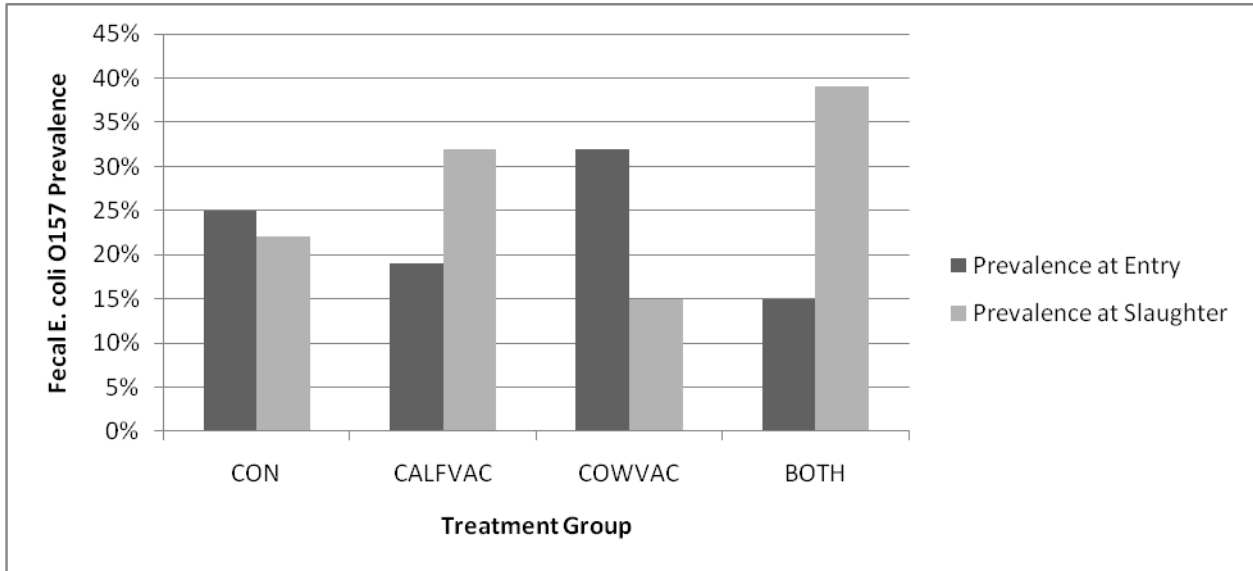**Figure 5.3:** Fecal *E. coli* O157:H7 prevalence in feedlot pens by calf treatment at feedlot entry and at slaughter.