EQUINE SALMONELLOSIS—MOLECULAR EPIDEMIOLOGY OF CLINICAL ISOLATES AND THE EFFECT OF ANTIBIOTICS ON THE CECAL MICROENVIRONMENT WITH PARTICULAR REFERENCE TO SHORT-CHAIN FATTY ACIDS AND THE SALMONELLA PLASMID VIRULENCE (*spv*) GENES

By

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I would like to dedicate this work to my family, Mom, Dad, Christopher, Alexis and Carissa—you define me. Without you, I am nothing, and cannot imagine my life in your absence. You have made me a better daughter, student, teacher, scientist, wife, mother, friend, and human being. You are my universe, and this work is just as much yours as it is mine.

I also dedicate this dissertation to every animal that has made the ultimate sacrifice in the name of research. Though it was not by choice, your gifts were never taken for granted, nor will they ever be forgotten.

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ABBREVIATIONS

%	percent(age)
+	positive
–	negative
ΔpH	pH gradient
≈	almost equal to
°C	degrees Centigrade (Celsius)
°F	degrees Fahrenheit
95%CI	95% confidence interval
A	acetate
AAD	antibiotic-associated diarrhea
ADH	test for arginine dihydrolase, red/orange = +
ADP	adenosine diphosphate
AMI	amikacin
AMOX	amoxicillin-clavulanic acid
AMP	ampicillin
AMY	amygdalin fermentation/oxidation test, yellow = +
ARA	arabinose fermentation/oxidation test, yellow = +
asd	aspartate semialdehyde dehydrogenase
ASP	acid shock protein
ATP	adenosine triphosphate
ATR	acid tolerance response
B	butyrate
BHI	brain heart infusion
bp	base pairs
C.	*Clostridium*
$CaCl_2$	calcium chloride
CEC	competent *Escherichia coli*
CEF or CEFA	cefazolin
CEFZ	ceftazidime
cfu	colony forming units
CH_4	methane
CHLP	chloramphenicol
CIT	test for citrate utilization, blue-green/blue = +
CLIN	clindamycin
cm	centimeter
CO_2	carbon dioxide
COD	cause of death

CON	control
df	degrees of freedom
DMC	direct microscopic count
DNA	deoxyribonucleic acid
dNTP	DNA nucleotides (A,C,G,T)
DOA	dead on arrival
DOX	doxycycline
E.	*Escherichia*
e.g.	for example
EB	ethyl butyrate
EDTA	ethylene diamine tetra acetic acid
ENRO	enrofloxacin
ERYT	erythromycin
et al.	and others
euth.	euthanatized
ex vivo	*outside the living body*
FOS	fructo-oligosaccharides
FUO	fever of unknown origin
g	grams
g	gravity, 10^{-11} N.m/s^2
GDUD	gastro duodenal ulcer disease
GEL	gelatinase production test, diffusion of black = +
GENT	gentamicin
GLU	glucose fermentation/oxidation test, yellow = +
gyr	gyrase
h	hour(s)
H_2	hydrogen
H_2O_2	hydrogen peroxide
H2S	test for hydrogen sulfide production, black = +
HCl	hydrochloric acid
HE	Hektoen-Enteric agar
i.e.	that is
IACUC	Institutional Animal Care and Use Committee
IB	isobutyrate
ICH	iodochlorhydroxyquin
IM	intramuscular
IMIP	imipenem
in vivo	*inside the living body*
IND	test for indole production, red = +
INO	inositol fermentation/oxidation test, yellow = +
IV	intravenous
IVA	isovalerate
kb	kilobase(s)
kg	kilogram
kV	kilovolts

l or L	liter
lb	pound (weight)
LB	Luria-Bertani
LBN	Luria-Bertani (sodium)
LDC	test for lysine decarboxylase, red/orange = +
LI	large intestine
log_{10}	logarithm base 10
M	molar
m	meters
M	molar
M9	minimal media
MAN	mannitol fermentation/oxidation test, yellow = +
MDa	megadaltons
MEL	melibiose fermentation/oxidation test, yellow = +
mg	milligram
mg/kg	milligrams per kilogram bodyweight
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulfate
MIC	minimum inhibitory concentration
min	minutes
ml	milliliter
mM	millimolar
mm	millimeters
mmol	millimoles
MOPS	morpholinepropanesulphonic acid (buffer solution)
MQMFK	modified Qiagen Midi Filter Kit for plasmid analysis
mRNA	messenger ribonucleic acid
N	normal
N/A	not applicable
NaCl	sodium chloride
NAHMS	National Animal Health Monitoring System
NAL	nalidixic acid
Nal^r	nalidixic acid resistant
NaOH	sodium hydroxide
NAX	ceftiofur sodium
ND	none determined
NG	nasogastric
NITR	nitrofurantoin
No.	number
NVFA	non-volatile fatty acids
ODC	test for ornithine decarboxylase, red/orange = +
ONPG	test for beta galactosidase, yellow = +
OX	oxidase test, violet = +
P	propionate
PBS	phosphate buffered saline

PCR	polymerase chain reaction
PEN	penicillin
PF	pelvic flexure
PFGE	pulsed field gel electrophoresis
pg	picograms
PGMAA	pH-gradient mediated anion accumulation
pH	negative logarithm of hydrogen ion concentration
pKa	negative logarithm of the acid dissociation constant Ka
PMN	polymorphonuclear leukocyte
PO	*per os* (orally)
ppm	parts per million
PRAS	pre-reduced anaerobically sterilized
q	every
QBT	Equilibration Buffer (Qiagen Midi Filter Kit)
QC	Wash Buffer (Qiagen Midi Filter Kit)
QF	Elution Buffer (Qiagen Midi Filter Kit)
R plasmid or factor	resistance plasmid or factor
RFLP	restriction fragment length polymorphism
RHA	rhamnose fermentation/oxidation test, yellow = +
RIF	rifampin
rpm	revolutions per minute
rpoS	alternative sigma factor (referring to the gene)
rpoS	alternative sigma factor (referring to the protein)
RT	room temperature
s	second(s)
S.	*Salmonella*
SAAAD	Salmonella-attributed antibiotic-associated diarrhea
SAC	sucrose fermentation/oxidation test, yellow = +
SC	small colon
SCFA	short-chain fatty acid
SD	standard deviation
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
SI	small intestine
SOR	sorbitol fermentation/oxidation test, yellow = +
SPF	specific pathogen free
spp.	bacterial species
spv	Salmonella plasmid virulence (referring to the gene)
spv	Salmonella plasmid virulence (referring to the protein)
subsp.	sub-species
TBE	tris-borate EDTA
TDA	test for deaminase, brown/red = +
TE	tris-EDTA
TET	(oxy)tetracycline
TMP or TMPS	trimethoprim sulfamethoxazole

TNTC	too numerous to count
TSP or TP	total serum protein or total protein
U	units
URE	test for urea hydrolysis, red/orange = +
V	valerate
v/v	volume per volume
var.	variant (serovariant)
VFA	volatile fatty acid
VMTH	Veterinary Medical Teaching Hospital
VP	Voges-Proskauer test for acetoin, pink/red = +
w/v	weight per volume
wt.	weight
χ	bacterial strain

Abstract of Dissertation Presented to the Graduate School
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Major Department: Veterinary Medicine

Antibiotic-associated diarrhea (AAD) is a common and potentially fatal disorder in horses and is often attributable to *Salmonella* spp. Disturbances in anaerobic microflora are thought to cause altered intestinal levels of bacteriostatic short-chain fatty acids (SCFA). Salmonella virulence plasmid (*spv*) genes are reported to increase ability of *Salmonella* to grow in organs outside the gastrointestinal tract. Horses treated with intravenous oxytetracycline (TET), oral trimethoprim-sulfamethoxazole (TMPS), and intramuscular ceftiofur (NAX) had significant differences in concentrations of seven individual cecal SCFA with TET having the most significant effects, followed by TMPS and NAX. No differences were detected in cecal protozoal counts, total culturable cecal anaerobes, or cecal pH compared to untreated horses.

Epidemiological techniques were used to investigate 106 cases of salmonella infection in horses at a veterinary teaching hospital over 2 years. Total mortality was

36.5%. Plasmid profiles, *spv* gene analysis, serovar, and antibiotic sensitivity data were recorded for all isolates. Odds ratios predicted an increased risk of a fatal outcome in horses younger than 4 years of age (3.3 times), horses infected with group B salmonellae (15.7 times relative to group D), and horses whose salmonella isolate possessed the *spv* genes (12.3 times). Extra-intestinal salmonella isolates were 12.2 times more likely to contain the *spv* genes. The majority of large plasmids in salmonella serovars isolated from horses were not virulence plasmids, but likely antibiotic resistance plasmids (3/3 tested transferred multiple resistances). This information suggests that the *spv* genes may play a similar role in horses as they do in humans, mice, and calves: to potentiate systemic infection after gastrointestinal infection.

Sterile-filtered cecal liquor from horses treated with ceftiofur or trimethoprim-sulfamethoxazole increased the *in vitro* anaerobic growth rates of *Salmonella* relative to plain media, and slightly more than untreated control horses cecal liquor. *Salmonella* grew equally as well (but much slower than NAX or TMPS) in TET treated horses cecal liquor and plain M9 medium. The SCFAs acetate, butyrate, and propionate, added to M9 minimal medium at 30mM or 100mM, exhibited a dose-dependent inhibition of anaerobic salmonella growth that was not attributable to the *spv* genes, with propionate 100mM > butyrate 100mM > acetate 100mM ≈ propionate 30mM > butyrate 30mM > acetate 30mM.

CHAPTER 1
INTRODUCTION

The Genus *Salmonella*

The Bacteria

The first mention of the yet-to-be-named genus *Salmonella* was a report in 1880 on a "typhoid bacillus" observed in the spleen and mesenteric lymph nodes of a fever patient.[1] A second organism discovered at approximately the same time, which failed to agglutinate in serum from typhoid patients, was designated "bacille paratyphique." The first documented cases of salmonellosis in animals were described by Salman and Smith in 1886 of swine affected with hog cholera. This bacterium was later designated *S.* Choleraesuis, and the genus eventually named after the former.[1]

Salmonellae are gram-negative members of the family Enterobacteriaceae. As of August 2002, the genus is represented by 2,523 distinctive serovariants (serovars)[2] of flagellated, facultatively anaerobic bacilli.[3] Salmonellae are speciated and sub-characterized by their O (LPS), H (flagellar), and Vi (capsular) antigens. O antigens are located on the surface of the outer membrane and are determined by specific polysaccharide sequences. H antigens are expressed on flagella, and they are composed of the proteins called flagellin. H antigens are biphasic and occur in either or both of two forms, phase 1 and phase 2. The bacteria are capable of switching from one phase to the other depending on environmental pressures.[1] Vi is a unique antigen in that it overlays the O antigen and is present in a limited number of serovars, the most important being *Salmonella* Typhi, a host-adapted serovar of humans.[4;5] These bacteria are stable and

ubiquitous in the environment, and they are capable of colonizing and infecting nearly all higher species, although some serovars are known to have host preferences as well as syndrome phenotypes.

Animal Models of Disease

Non-typhoidal salmonellae are global enteric pathogens of humans and other vertebrates, and decades of research have been devoted to the epidemiology, pathogenesis, diagnosis, control, and effective treatment of salmonellosis. To date, the most economic and thoroughly characterized animal model of salmonellosis has been the mouse. Salmonella infection in the mouse typically produces a syndrome of fever and bacteremia. Until recently, it was thought that the mouse species did not develop enteritis secondary to orogastric inoculation with *Salmonella*,[6] which is typical of the pathogenesis in most mammals, including humans and horses.[7] In humans, cows, and horses, the inflammatory reaction of the gastrointestinal tract is predominantly neutrophilic, while in the mouse the mononuclear cell is the principal inflammatory cell.[8] The serovar- and route-dependant clinical response to experimental infection in the mouse is different to that of most other vertebrates. Watson et al. showed that the cellular route of intestinal invasion is different between mice and calves, with M cells and Peyer's patches being the preferred targets in the murine host in contrast to enterocytes in calves.[9] This creates difficulty in the extrapolation of experimental data from mice to larger mammals. Several alternative models to human gastrointestinal salmonellosis have been developed, using the calf[10] or pig,[11;12] but these are expensive, logistically difficult to maintain, and carry significant animal welfare concerns.[4;6] In 2003, a newly proposed mouse model of enteric salmonellosis was described and successfully tested by Barthel et al. in Belgium.[13] This model more closely approximates the neutrophilic inflammatory infiltrate seen in

response to the bacteria, yet the mice still do not become diarrheic. Despite this, the model is still a step forward in terms of salmonella investigation on genetic, immunologic, and environmental levels, as there are readily available genetic knockout strains and immunohistologic media and protocols developed for the mouse species.

The Salmonella Virulence Plasmid

The term plasmid is used to describe autonomously replicating extrachromosomal DNA. This DNA is not critical to cell survival *in vitro* but can confer specific characteristics that allow the host cell to survive during adverse conditions or to cause disease.[14] Pathogenic salmonellae possess a collection of these attributes, called virulence factors.[15;16] These include factors that convey acid resistance, enhance the ability to invade non-phagocytic cells, elicit inflammation, support resistance to destruction by phagocytic cells, suppress the immune system of the host organism, enhance intracellular replication, and encode antimicrobial resistance. Several of these factors can be attributed to the presence of a large 50-100 kb plasmid, originally termed the "cryptic plasmid" as its purpose was unclear, but now described as a virulence plasmid.[17;18] The virulence plasmid of *Salmonella* has been characterized extensively in the mouse typhoid model and appears to be most important in the ability of the organism to multiply in systemic tissues after dissemination from the gastrointestinal tract.[19-21] Clinical significance of this virulence plasmid has been examined in several studies, and there remains disagreement regarding contribution of the virulence plasmid to bacteremia and replication in extra-intestinal tissues. Clinical isolate data from several human studies also agree with the murine model: virulence plasmids are more likely to be present in those isolates obtained from systemic sources such as blood, liver and spleen, compared to unrelated isolates obtained from feces.[22;23] Conflicting reports utilizing

comparable experimental methods have shown no causal relationship between bacteremia and presence of the virulence plasmid in humans.[24;25] Discordant results are also seen within the model using calves orally infected with *S.* Dublin. The virulence plasmid containing the *spv* genes was shown to be important in determining the severity of diarrhea in calves,[10] while other investigators demonstrated no influence of the virulence plasmid (as compared to naturally occurring plasmid-free, or plasmid-cured isolates) on enteropathogenesis either *in vivo*, or in *ex vivo* ligated ileal loop experiments.[26] This serovar-host-syndrome interrelationship is most certainly a confounding factor in determining the pathophysiologic importance of the salmonella virulence plasmid.

Salmonella Plasmid Virulence (*spv*) Genes

The plasmids of several serovars contain a 7.8-kb salmonella plasmid virulence (*spv)* region, which contains five genes (*spvRABCD*) that are highly conserved across the serovars that possess them.[10;20;27] Those serovars tend to be natural host-adapted salmonellae, including *S.* Dublin, *S.* Choleraesuis, *S.* Abortusovis, and *S.* Gallinarum-Pullorum, but have also been found in broad host range serovars such as *S.* Typhimurium, and *S.* Enteriditis.[10] The genes contained within that small region are sufficient to replace the virulence phenotype of the entire plasmid in animal systemic infection models.[27] *spvR* encodes a transcriptional activator of the LysR/Met R family of regulatory proteins and is transcribed independently from the four effector genes *(spvABCD)*. SpvR binds to the *spvR* and *spvA* promoters and directs transcription of itself and *spvABCD* during stationary phase growth.[28] The full significance of *spv* genes on bacterial pathogenicity is becoming more clear, and they have been associated with enhanced virulence in mouse systemic infection models,[20;29-30;31] as well as showing enhanced expression after invasion of both phagocytic and epithelial cells. [32;33] The *spv* genes are not necessary for

the bacteria to colonize the mouse gastrointestinal tract or invade mucosal cells to initiate a systemic infection.[20] They are also not required to survive in mouse secondary organs such as liver and spleen.[34] They have been shown, however, to accelerate proliferation of the organism in the reticuloendothelial system,[21] are essential to cause cytopathology in mononuclear cells,[35] and are associated with increased mortality in the calf model of oral infection.[10] A simplistic diagram showing the current opinion of how the *spv* genes are regulated in salmonella serovars is shown in Figure 1-1.

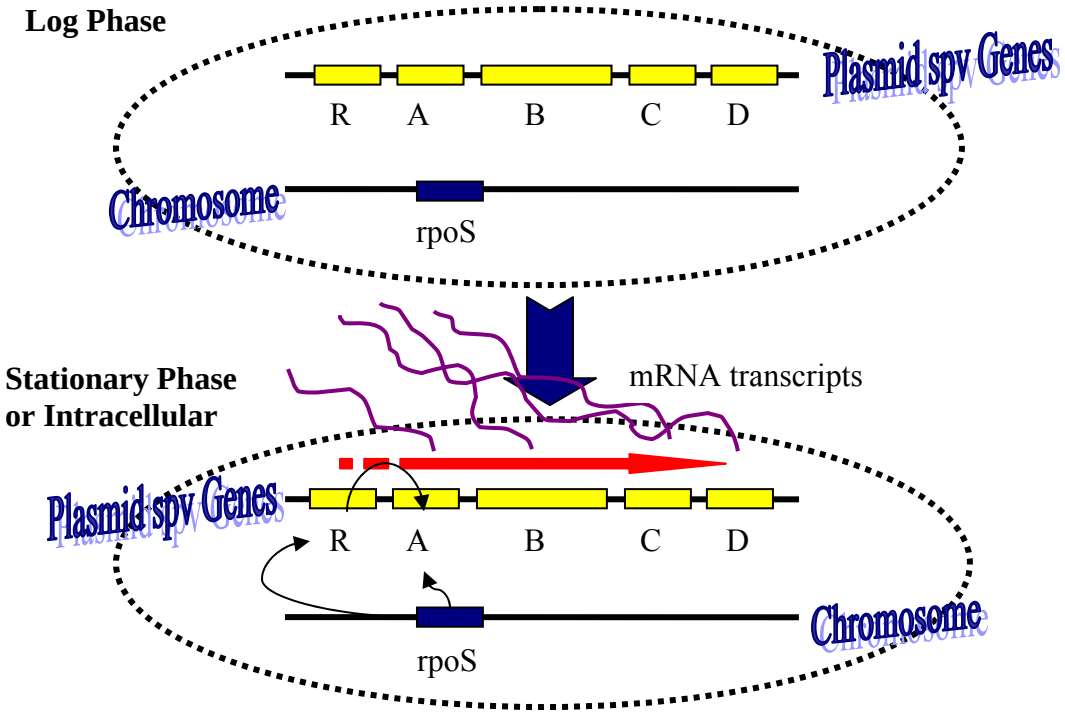

Figure 1-1. *spv* gene expression regulation is dependent on growth phase and cellular location in *Salmonella*

A significant proportion of salmonella serovars isolated from clinical cases of human[22] and bovine[23] diarrhea do not contain virulence plasmids and, therefore, the *spv* genes. It appears that the ability of the *spv* genes in *Salmonella* to cause or enhance pathology depends on other bacterial factors (e.g., chromosomal) as well as host factors.

The *S*. Enteriditis virulence plasmid containing the *spv* genes was placed into a plasmid-cured *S*. Dublin strain, and virulence was restored in a mouse model,[36] while the virulence plasmid from *S*. Dublin pSDL2 only variably transferred a virulent phenotype to serovars that did not commonly carry a virulence plasmid.[37] Although the *spv* genes are present and conserved across several serovars, many different syndromes and outcomes of infection have been clinically or experimentally observed within those subgroups. The work described in this dissertation attempts to analyze the role of the virulence plasmid and the *spv* genes in the pathogenesis and epidemiology of equine salmonellosis.[10;38]

Short-Chain or Volatile Fatty Acids and *Salmonella*

Salmonella spp. are enteroenvironmentally transmitted pathogens of humans and animals. It is therefore expected that during their life cycle they are exposed to extremes in temperature, oxygen availability, pH, osmolarity, nutrient availability, organic acid concentration, and presence of other bactericidal compounds such as reactive oxygen species. *Salmonella* have shown remarkable ability to sample their environmental conditions and use this information as a signal for growth, stasis, or expression of virulence factors. This ability is known as "quorum sensing" and is present in several opportunistic and/or pathogenic species of bacteria.[39] It has also been shown in *Salmonella* that the induction of a stress resistance response to one condition, (e.g., low pH) confers resistance to multiple stress conditions.[40] Ironically, the end-result of carbohydrate feeding to horses (instead of a complete forage-based diet) may actually be priming resident or transient salmonella organisms and extending their spectrum of resistance to organic acids and other stressors. This may indirectly predispose horses to development of salmonella-induced diarrheal disease by seeding their environment with

bacteria that are more virulent than their acid-susceptible or otherwise stress-naïve cohorts.

Short-chain fatty acids are normally found in relatively high concentrations in the forestomachs of ruminants,[41] the cecum and large intestine of all warm-blooded vertebrates,[42] and the crop, cecum, and large intestine of birds.[43] Acetate, butyrate, and propionate are typically found in the highest percentages, with smaller amounts of isomeric and variable sized carbon-chain compounds.[41] In a general sense, these acidic end products of anaerobic fermentation reactions help to keep the endogenous population of bacteria within the intestines at a stable level and discourage transient pathogens from becoming established. They can also be absorbed and function as an energy source for the host animal, or they can be directly utilized by colonocytes.

Antibiotic-Associated Diarrhea (AAD) in the Horse

Diarrhea is one of the most common and recognized side effects of antibiotic therapy in all species, especially the horse. Symptomatically, it can range from mild loss of fecal consistency to projectile liquid feces and/or intestinal pseudomembrane formation. A long-standing hypothesis suggests that disruption of the normal chemical and biological balance within the intestine is responsible for the development of colitis, either during or after the cessation of antibiotic therapy. This relationship may or may not be true in horses. In one case-control study, horses which had received parenteral or oral antibiotics were 40 times more likely to develop diarrhea than horses which had received no therapy.[44] Also, in a documented outbreak of salmonella diarrhea in a large hospital, horses that had received parenteral antimicrobial therapy were at 10.9 times greater risk of having *Salmonella* isolated from their feces than were matched cohorts not receiving antibiotics.[45] However, three longitudinal studies have demonstrated no clear

association between antibiotic administration and salmonella infection in horses.[46-48] Any antibiotic, given by any route, to any horse, for any length of time, has the potential to cause diarrhea,[49] though orally administered agents and those drugs having a biliary component to their metabolic-cycle pose a greater risk.[50] Oxytetracycline,[51-55] penicillin V and G,[56] aminopenicillins,[57] metronidazole,[56] lincosamides,[58-60] trimethoprim-potentiated sulphonamides,[61;62] third generation cephalosporins,[56] and macrolides[63] all have diarrhea as a reported side effect in the horse, though there are conflicting data for specific antibiotics (e.g., trimethoprim-potentiated sulphonamides).[49;51] The situation becomes pivotal in the equine species due to several factors, most importantly 1) the large capacity of the digestive tract, therefore the potential of enormous amplification and dissemination of the infectious agent into the environment, and 2) the intensive management of horse operations—with overly susceptible animals such as neonatal, geriatric, pregnant, and immunocompromised individuals often kept in direct contact with asymptomatic animals shedding *Salmonella*. From a therapeutic standpoint the horse also presents more unique challenges. First, the potentially large volume of fluid excreted per day is difficult to replace—oral and/or parenteral fluid therapy is the cornerstone of therapy in treatment of horses with large colon disease. Second, the horse is uniquely susceptible to many secondary complications of enterocolitis that in and of themselves could be as life-threatening as the diarrhea itself. The large bio-burden of gram-negative bacterial cell wall (endotoxin) contained within the adult equine gastrointestinal tract is more than adequate to cause severe disease or mortality should it gain access to the circulatory system. Third, it has been shown clinically as well as experimentally that horses can asymptomatically harbor and shed virulent organisms for

unpredictable amounts of time, either following acute infections or without previous illness[64;65] and the ability to positively identify a carrier animal based on appearance alone is impossible.

The Gastrointestinal Microenvironment

The Normal Flora

The terms "resistance to colonization" or "competitive exclusion" have been used to describe the passive ability of the gastrointestinal tract to keep pathogenic organisms from becoming established.[66;67] In humans, the anaerobic component of the commensal microflora has been determined to be primarily responsible for maintaining the colonization resistance toward pathogens.[68] Despite the multitude of potentially virulent organisms ingested on a continual basis, the innate functions of the intestinal microenvironment restrict a pathogens ability to attach, multiply, invade and cause disease. Intestinal anatomy and motility, mucosal epithelial and immune cells, the enteric nervous system, residential bacteria, protozoa and their by-products, and mucosal immunoglobulin all combine with digesta to comprise this effective barrier to pathogens.[69]

The predominant species and demographics of the bacterial population change with respect to the section of the intestine being colonized. Host diet, oxygen tension, pH, redox potential, and intestinal motility all determine the constitution of the normal intestinal flora, and even this may change on an individual or daily basis. Generally speaking, anaerobic bacteria significantly increase as a percentage of the total bacteria progressing aborally through the gastrointestinal tract.[70] These anaerobic bacteria are responsible for the breakdown of otherwise indigestible saccharide bonds and the production of SCFA and gases such as methane and carbon dioxide. Short-chain fatty

acids are also important food sources for the colonic mucosal cells and are used by the host organism as an energy source.[42]

Short-Chain Fatty Acids—Production and Intestinal Function

Short-chain fatty acids are bacterial by-products of fermentation reactions that occur in an anaerobic environment. Non-spore forming anaerobes are the principal facilitators of this process through the Embden-Meyerhof-Parnas pathway.[71] They have been studied extensively with respect to production sites, rates of appearance, and biological fate in many species.[41] SCFAs are important for development and proper function of the rumen, intestine, and mucosal epithelium. The SCFAs, methane, carbon dioxide , and hydrogen are the main end-products of anaerobic bacterial fermentation of carbohydrates, while the branched-chain SCFAs are breakdown products of proteins and are produced independently of the others.[72;73]

Table 1-1. Short-chain fatty acid chemical formulas and common names

Chemical Formula	Common Name
CH_3-COOH	Acetate
CH_3-CH_2-COOH	Propionate
CH_3-$(CH_2)_2$-COOH	Butyrate
CH_3-CH(-CH_3)-COOH	Isobutyrate
CH_3-$(CH_2)_3$-COOH	Valerate
CH_3-CH(-CH_3)-CH_2-COOH	Isovalerate
CH_3-$(CH_2)_2$-CO_2CH_2-CH_3	Ethyl butyrate (ethyl butanoate)

Herbivores (especially the ruminants) obtain significant amounts of energy (up to 70-80% of daily maintenance) from the absorption and metabolism of SCFAs, which are produced via bacterial breakdown of dietary lignin, pectin, cellulose, and hemicellulose.

SCFA production and anaerobic respiration pathways in the ruminant with substrates and intermediate compounds are shown in Figure 1-2, modified from Van Soest.[74]

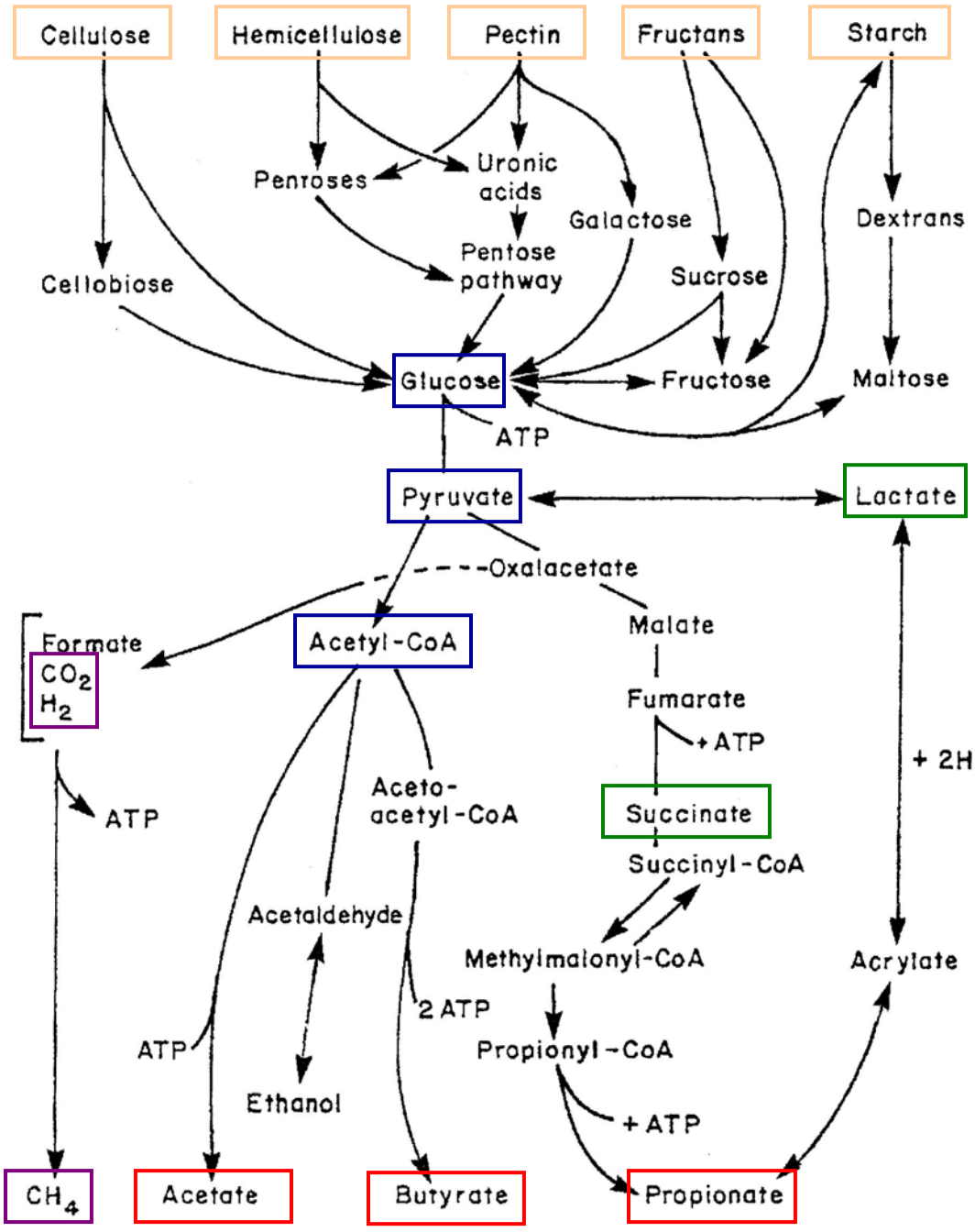

Figure 1-2. Polysaccharide metabolism and SCFA production pathways in the rumen. Modified from Van Soest.[74] Tan boxes indicate substrate, red boxes indicate SCFAs, green boxes indicate NVFAs, blue boxes indicate important intermediate compounds, and purple boxes indicate accumulated end-products.

Humans and other monogastric species such as the dog obtain much less energy (6-9%) from the utilization of endogenously produced SCFAs.[42] Additional sources of substrate include sloughed intestinal epithelial cells, blood, mucins, digestive enzymes, and miscellaneous resistant starches.[73] Figure 1-3 depicts the interrelationship between anaerobic microbial function and the products of fermentation.

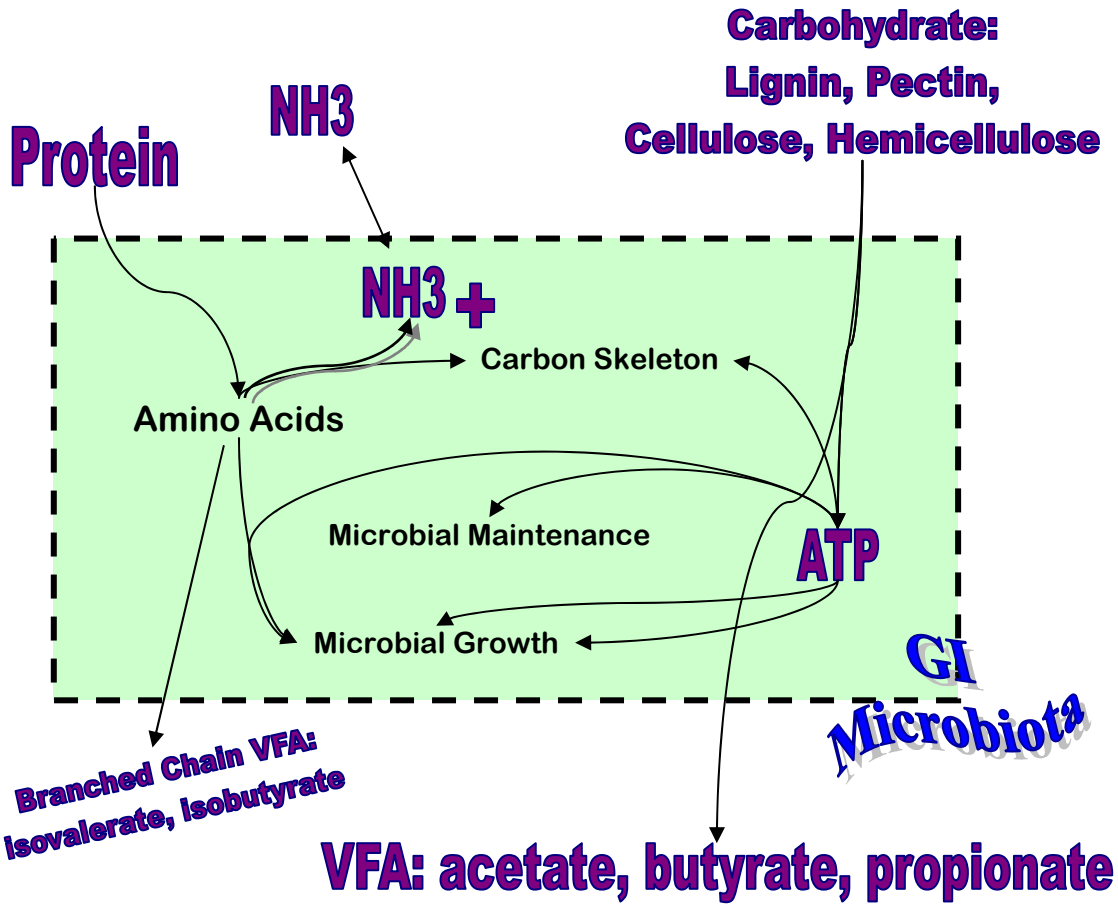

Figure 1-3. Diagram of major gastrointestinal microbial digestive and energy functions, nitrogen and carbon recycling, and SCFA production

In addition to local consumption, SCFAs are shuttled directly into the portal circulation for peripheral and hepatic metabolism. Short-chain fatty acid contributions to maintenance energy requirements of the host range from less than 10% in humans and

dogs up to more than 80% in the ruminant and large-intestine fermenters such as the horse.[75] Those SCFAs are utilized by the rumen or ceco-colonic mucosal epithelial cells as an energy source and also influence intestinal blood flow and water and electrolyte secretion and absorption.[73] Short-chain fatty acids are intimately involved in the proper function and regulation of the terminal digestive processes as shown in Figure 1-4.[73;75;76] "Colonic starvation" or "nutritional colitis" are phrases used to describe a diarrhea seen in patients fed either total parenteral (intravenous) nutrition or enteral tube formulas low in fiber.[77] The hypothesis involves decreased SCFA production in the colon, with the colonocytes becoming malnourished, leading to abnormal water and sodium absorption. It was also shown that deranged fermentation in the large intestine in response to antibiotic administration did not necessarily predict the development of diarrhea, though all patients that developed antibiotic-associated diarrhea had fermentation abnormalities.[78] This suggests that purely the absence or impairment of SCFA synthesis is not enough to cause diarrhea but may be an essential predisposing condition.

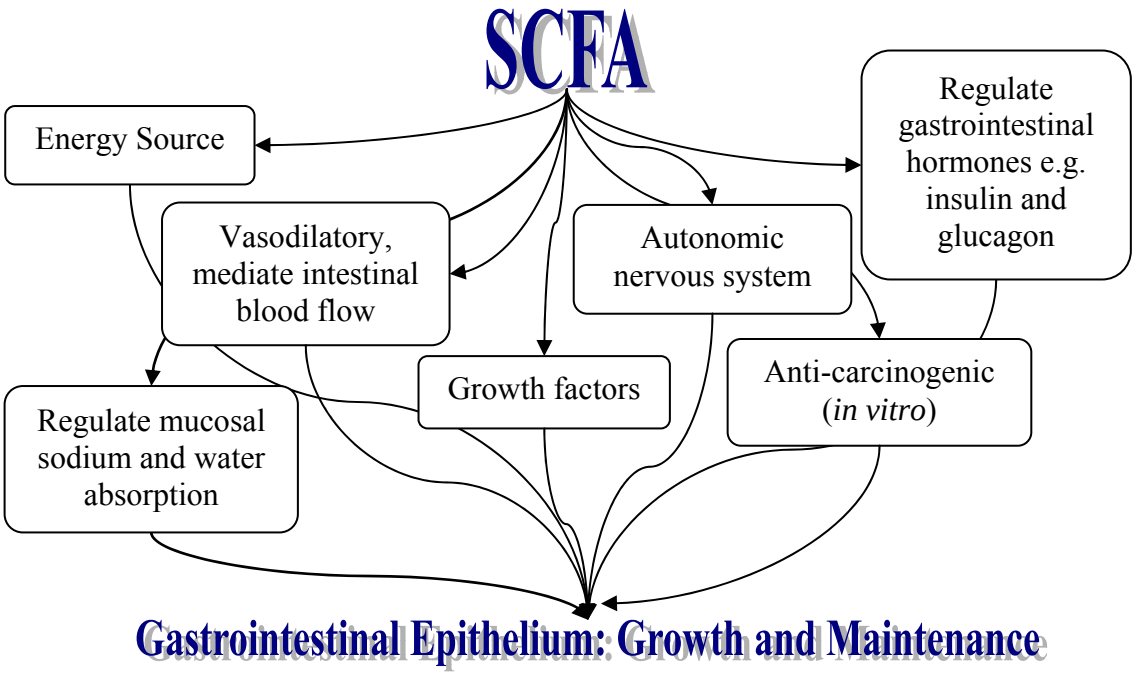

Figure 1-4. Summary of potential enterotrophic effects of SCFA

Effects of Antimicrobial Therapy: Dysbacteriosis[61]

The incidence of AAD is estimated to be between 5-25% of all humans receiving antibiotics, though patient risk group, type of antibiotic, and route of administration will affect true prevalence.[79] Current hypotheses suggest that the gastrointestinal side-effects of antimicrobials are manifested through disruption of autochthonous anaerobic flora, particularly *Bacteroides, Bifidobacterium, Lactobacillus* and *Streptococcal* spp. Anaerobic bacteria are critical for fermentation of carbohydrates and production of SCFAs, and it is these acids that are believed to have natural and regulatory bactericidal and bacteriostatic properties against enteric commensals as well as pathogens.[80] Several investigators have reported significant disruptions in anaerobic flora and SCFA concentrations in animals, humans, xeno-transplanted flora models, and *in vitro* colon simulation systems treated with antimicrobials.[81-88] Intestinal colonization and increased multiplication rates of *S.* Typhimurium in response to streptomycin treatment in mice

were associated with decreased concentrations of fecal SCFAs and increased luminal pH.[89] *In vitro* supplementation of SCFA to the cecal contents of treated animals inhibited salmonella growth in this model.[80] Further studies have shown that this protection may be conferred by specific SCFAs as elevated concentrations of propionic or formic acid added to feed conferred significant protection against *S.* Typhimurium cecal colonization in chicken hatchlings.[90]

Another theory links the etiology of AAD to the reduction or disappearance of SCFAs. These acids are regulators of sodium and water uptake in the colon, and their absence causes an indirect accumulation of sodium and water in the intestinal lumen.[91] Sodium is a potent cellular osmolyte which draws more water across membranes and into the lumen, causing increases in fecal water content. This theory does not adequately account for the magnitude of diarrhea seen in some AAD patients, but it could easily be an initiator or contributor to pathogenesis.

Alternative popular assumptions of the pathogenesis of AAD include unchecked overgrowth of *Clostridium difficile* (especially in human neonates) with production of potent entero- and cyto-toxins or the vacating of attachment sites or toxin receptors normally occupied by host commensal bacteria.[92] *C. difficile* has been identified as a pathogen in equine AAD.[93]

Specific Aims

The specific aims of the reported studies were to:

- Collect *Salmonella* spp. isolates from clinical cases of equine salmonellosis and from normal horses.
- Examine case history and collect relevant host data (age, breed, gender, presenting disease, risk factors, biochemical profiles, antimicrobial susceptibilities, treatments) for all salmonella isolates.

- Determine if the salmonella isolates carried large plasmids.
- Determine if the salmonella isolates carried *spv* genes.
- Determine the cecal SCFA concentrations, luminal pH, total culturable anaerobic bacterial counts, and protozoal counts of horses before and after treatment with selected antibiotics.
- To examine *spv*+ and *spv*- salmonella isolates in terms of growth rate during anaerobic culture in nutrient broth supplemented with sterile-filtered cecal contents from antibiotic-treated versus non-treated horses.
- To examine *spv*+ and *spv*- salmonella isolates in terms of growth rate during anaerobic culture in nutrient broth adjusted to the mean luminal cecal pH and supplemented with individual SCFAs normally found in horse cecal liquor.
- To examine plasmid containing *spv*- isolates for antibiotic resistance determinants located on the plasmids.

Hypotheses

- Large plasmids in salmonella isolates are directly correlated with presence and type of disease.
    - Isolates from normal horses will not have plasmids.
    - Isolates from cases of diarrhea will variably contain plasmids.
    - Isolates from systemic cases will always contain plasmids.
- Salmonella isolates with large plasmids will also contain *spv* genes on those plasmids.
- The administration of repeated doses of commonly used antimicrobial agents to healthy horses will reduce the total culturable anaerobic bacterial population of the cecum, resulting in a reduction in the concentration or disruption of relative proportion of SCFAs and an increase in luminal pH.
- The administration of antimicrobial agents to healthy horses will reduce the numbers of cecal protozoa.
- Sterile-filtered cecal contents from horses that were not treated with antibiotics will inhibit the growth of *Salmonella* compared to sterile-filtered cecal contents from animals that received antibiotics in a *spv*-dependant manner.
- Nutrient broth containing individual SCFA will inhibit growth of *Salmonella* under anaerobic conditions in a dose-dependant and *spv*-dependant manner.

- Large plasmids in salmonella isolates that do not contain *spv* genes are likely antibiotic resistance plasmids.

CHAPTER 2
SALMONELLA IN HORSES—DISEASE DEFINITION AND GENERAL AND MOLECULAR EPIDEMIOLOGY

Background

Disease Overview

In spite of pharmacological and therapeutic advances, diarrhea in the adult horse continues to be one of the most challenging and frustrating medical syndromes facing the equine veterinarian. *Salmonella* spp. are one of the primary etiological agents of equine diarrhea, although a large number of diarrhea cases will progress or resolve without a definitive diagnosis. Salmonella infection of horses is not limited to the intestinal tract. There is potential for bacteremia, particularly in foals, with seeding of synovial structures, bone, lung, umbilical remnants, brain and meninges, liver, and kidneys. Salmonellosis can quickly become a financial disaster for the intensively managed horse farm or equine hospital given the potential copious nature of contaminating feces produced by one diarrheic adult horse, along with the environmental persistence of the organism. There are also serious human health issues regarding the zoonotic potential from treating and handling these animals.

There are four recognized clinical syndromes of salmonella infection in horses: 1) an asymptomatic carrier or latent state;[64] 2) a severe and sometimes fatal fibrinonecrotic enterotyphlocolitis; 3) bacteremia—with or without secondary foci of infection; and 4) pyrexia, depression, and leukopenia without diarrhea—similar to the "enteric fever" syndrome seen in humans infected with *S.* typhi.[94]

Prevalence

Excretion of *Salmonella* into the environment commonly occurs in horses without signs of enteric disease. This may be an animal that has recently recovered from infection, an animal that has acquired bacterial organisms via ingestion of contaminated feed, water, or bedding material and is simply a transient portal, or a chronically colonized host that has adapted a traditionally pathogenic relationship into a commensal one. It is the apparently healthy, but chronically colonized animal that represents the greatest danger to the population. Estimates vary widely depending on the population sampled and the diagnostic methodology used of the percentage of the horse population that is shedding *Salmonella*. The recent National Animal Health Monitoring System (NAHMS) survey reported that 0.8% of resident horses sampled in the US excreted *Salmonella* in their feces.[95] The majority of horses in this survey had normal fecal appearance at the time of sampling, although 2.1% had loose or watery feces. The prevalence of salmonella shedding was not higher in animals that had received antibiotics within the past 30 days.

Reported Risk Factors for Salmonella Infection

It has been noted that horses have an increased risk of developing salmonella-induced diarrhea after certain "stressors" have been placed on them, including but not limited to transportation,[96] hospitalization,[47] nutritional excess or deficiency,[54] dietary change,[97] colic—especially large colon impaction,[47;98] nasogastric intubation,[44;45] debilitating injury or illness, antibiotic therapy,[44;45;48] parturition, weaning, surgery, anesthesia, or anthelmintic therapy.[99;100] The challenge inoculum for these "at risk" individuals can be up to 100-fold smaller than for non-stressed and immunocompetent cohorts.[94;101;102] It is for these reasons that horses admitted to veterinary hospitals, even

on an outpatient basis, are highly susceptible to infection. The populations at greatest risk are those horses with gastrointestinal diseases admitted to referral hospitals for medical or surgical therapy.[44;45;101]

Salmonella Serovars Associated with Equine Infection

Approximately 60% of known salmonella serovars belong to the *S. enterica* subsp. *Enterica* group and within this group the O-antigen designations A, B, C_1, C_2, D, & E account for 99% of all warm-blooded animal infections. All O-antigen groups have been isolated from horses, but groups B, D, and E are the most common.[101] Commonly, phenotypic and molecular analyses are married to form the most accurate picture of an isolate as possible. Analysis of antimicrobial susceptibility, serogroup, serovar, phage type, plasmid profile, ribotype, or restriction endonuclease examination allows more specific identification of salmonella organisms. Newer and more precise methods of distinguishing salmonellae include polymerase chain reaction (PCR) fingerprinting, multiplex PCR, pulsed-field gel electrophoresis (PFGE),[103] restriction fragment length polymorphism (RFLP), IS200 typing,[104] and real-time PCR.[105] PCR has demonstrated itself to be one of the most sensitive and expedient methods of detecting *Salmonella* spp. in equine fecal samples, though culture is still the most cost-effective and widely available.[106] This is most helpful from an epidemiologic and control standpoint or for biologic surveillance programs. Though the treatment does not vary between serovars, specific identification could help in cases of outbreak, treatment failure, or when more than one strain of *Salmonella* is suspected.

Salmonella serovars frequently reported isolated from horses over the last 40 years include Agona, Anatum, Arizonae, Enterica, Enteriditis, Heidelberg, Infantis, Krefeld, London, Miami, Muenchen, Muenster, Newport, Oraneienburg, Rubislaw, Saintpaul,

Senftenberg, Thompson, Typhimurium, and Typhimurium var. Copenhagen.[46;47;95;97;99;107-111] Almost all serovars of *Salmonella* infecting horses are non-host adapted strains,[101] with the exception of Abortusequi, which does not cause gastrointestinal disease, but rather early abortion in mares and systemic sepsis in newborn foals.[112] Horses are also susceptible to some of the normally host-adapted serovars of other species such as *S.* Dublin (bovine) and *S.* Choleraesuis (porcine).[110]

The herbivorous and gregarious nature of horses makes them efficient dispersal agents as well as susceptible recipients for the entero-environmental cycling of *Salmonella*. Compounding this issue, salmonellae are ubiquitous and environmentally resistant and can remain infectious in fecal material for years under the appropriate conditions.[113]

Role of Microbial Virulence Factors in Equine Salmonella Infection

Specific virulence factors that mediate systemic or gastrointestinal salmonella infections in horses have not been extensively studied. Likely this is due to reluctance or difficulty in using the horse as a model of disease. Retrospective studies examining isolates obtained from clinical cases of salmonellosis have been published, but investigators focused on more epidemiological than molecular techniques of comparison.

Disease Prevention—Diet, Probiotics, Immunity

Methods utilized by veterinarians to decrease the morbidity and mortality of salmonella infection in horses have either limited scientific basis or are applied based on results obtained from other species. Very little information is available on specific preventative strategies or therapies once clinical signs become evident.

Fructo-oligosaccharides (FOS) have been utilized extensively as feed additives in the poultry and companion animal industries for many years. They exert their effects by

increasing the amount of fermentable carbohydrate that reaches the large intestine, which can be acted upon by the bacterial population. This in turn raises the concentrations of organic acids and drops the pH, which presents an inhospitable environment to pathogenic species.[114]

"Direct-fed microbial" and "competitive exclusion" are terms used frequently in the poultry industry to describe a practice and physiologic phenomenon of directly feeding or facilitating the establishment of a desirable microbial population in order to discourage colonization by an undesirable one, typically *Salmonella*. Transfaunation via fecal slurry or cecal or colonic contents from a recently euthanatized or cannulated horse are techniques used in a hospital situation to re-establish commensal protozoa and bacterial flora in horses with diarrhea. Enemas of slurried fecal material from normal individuals, have been shown quite effective at treating or preventing antibiotic associated diarrhea in humans, but are unlikely to be beneficial in horses due to anatomical differences.[115] A commercial probiotic preparation is available for use in horses (Probios® Equine One Gel, Chr. Hansen BioSystems), however clinical efficacy data of this type of product in horses is limited. In a prospective study of hospitalized horses neither of two commercial probiotic formulations had any effect on salmonella shedding, incidence of diarrhea, or length of hospitalization following abdominal surgery.[98] A recent prospective study examining the probiotic potential of *Lactobacillus rhamnosus* strain GG in horses failed to show efficient colonization of the adult gastrointestinal tract unless extremely large doses were administered, though foals were more consistently and efficiently colonized.[116] These conflicting results should be further investigated, as human evidence is strongly in favor of the use of direct-fed microbials in the prevention and management

of antibiotic-associated diarrhea or other diarrheas attributed to dysbacteriosis. Significant benefit could be obtained from a small daily dose of orally administered bacteria during periods of increased susceptibility to salmonellosis, such as during extended travel or preceding and concurrent with antibiotic administration.

Immunity to *Salmonella* is dependent on a combination of cell-mediated recognition and destruction by activated granulocytes, as well as an antibody driven humoral response. *Salmonella* antibody-containing equine plasma products are commercially available. These products are almost exclusively used for the treatment of systemic salmonellosis in foals, or as preventative therapy in foals with failure of passive transfer in areas with a history or high prevalence of disease. These products are usually cost prohibitive for use in adult horses, and more importantly, are serovar specific, thus providing no cross protection to the significant number of other serovars able to infect horses. Mucosal immunization of horses with mutant strains of *Salmonella* rendered non-pathogenic has also been examined. Sheoran et al. demonstrated strong production of *S.* Typhimurium specific mucosal IgA in jejunal, nasal, and vaginal compartments after intra-nasal vaccination of ponies with a Δ*cya* Δ*crp-pabA* mutant of *S.* Typhimurium.[117] This strain is attenuated for virulence by deletion of the genes necessary for adenylate cyclase production (*cya*) and the cyclic AMP receptor protein (*crp*). This live vaccine did not cause any signs of disease, was not shed in the feces, nor was it transferred to cohabitated non-vaccinates. Mucosal specific antibody is an attractive first line of defense against enteric pathogens, and exploitation of the gastrointestinal mucosal immune system in the horse is attractive in terms of prevention and protection.

Disease Treatment

The treatment of salmonella infection is controversial and dependent on several factors, including severity of disease, immune status, metabolic state, age, concurrent malignancy, drug cost, drug availability, side-effects, and the presence of colonizable foci (e.g., implanted materials, catheters). Conventional antibiotic therapy of uncomplicated salmonella gastroenteritis in human beings is often not efficacious and may actually prolong the convalescent phase and/or extend the length of time that *Salmonella* is shed from the feces.[118-120] Even antibiotics preferred for the directed therapy of *Salmonella* in horses and humans (e.g., fluoroquinolones) have not had any scientifically reproducible or predictive effects on fecal carriage post-infection. Post-convalescent shedding is an important salmonella-related morbidity issue facing the equine practitioner. Contamination of the environment with persistent, virulent, and potentially antibiotic resistant bacteria is a cause for concern in a horse facility, especially a veterinary hospital. Outbreaks of nosocomial salmonellosis have resulted in institutional shut-downs world-wide.[109;121-128] In these circumstances antibiotic therapy of clinically silent or uncomplicated cases would be useful if the period of environmental contamination could possibly be shortened, thereby limiting exposure of other animals while the facility is depopulated and disinfected.[129]

The Salmonella Virulence Plasmid

Clinical isolate data from several human studies agrees with the murine model of salmonellosis: virulence plasmids are more likely to be present in those isolates obtained from systemic sources such as blood, liver, and spleen, compared to unrelated isolates obtained from feces.[22;23] Conflicting reports utilizing comparable experimental methods have shown no causal relationship between bacteremia and presence of the virulence

plasmid in humans.[24;25] Discordant results are also seen within the model using calves orally infected with *S.* Dublin. The virulence plasmid containing the *spv* genes was shown to be important in determining the severity of diarrhea in calves,[10] while other investigators demonstrated no influence of the virulence plasmid (as compared to naturally occurring plasmid-free or plasmid-cured isolates) on pathogenesis either *in vivo* or in *ex vivo* ligated ileal loop experiments.[26] This serovar-host-syndrome interrelationship is most certainly a confounding factor in determining the pathophysiologic importance of the salmonella virulence plasmid.

Salmonella Plasmid Virulence (*spv*) Genes

Function of the *spv* genes

The function of the *spv* genes in *Salmonella* has been a focus of investigation for many years. Highly conserved genomic elements should theoretically be important to the survival and host-to-host transmission of pathogenic bacterial species. Of the entire *spv* locus, it has been shown that only *spvB* and *spvC* are essential for full virulence in the mouse model of subcutaneous infection,[31] and more recently, that *spvB* was required for cytotoxic pathology (progressive detachment of adherent cells, vacuolization) and apoptosis after phagocytosis by human monocyte-derived macrophages.[35] The apparent accelerated growth of *spv* positive strains (as compared to *spv* negative) and their ability to cause systemic disease may actually be an extension of their ability to survive and travel within macrophages to these sites. A summary of the current understanding of the molecular and functional information regarding the *spv* genes can be found in Table 2-1.

Table 2-1. *spv* gene characteristics

Gene	Activity	Protein Localization	Significance
spvR	Transcriptional activator of *spvR* and *spvABCD*	Cytoplasm	Positive regulator (promoter) of itself and the other *spv* genes
spvA	unknown	Outer membrane[130]	Unknown, mutations do not affect virulence in mouse intraperitoneal infection model.
spvB	ADP-ribosyltransferase	Cytoplasmic and transported out of cytoplasm, small amounts in inner membrane[130]	Essential for full virulence (mouse model).[31] Effector protein causing depolymerization of actin cytoskeleton within macrophages. Inhibition of phagolysosome fusion.
spvC	unknown	Cytoplasm	Essential for full virulence (mouse model)[31]
spvD	unknown	Exported outside of cell	Mutations attenuate virulence (mouse model)[131]

Significance of the *spv* genes

The role of *spv* genes in equine salmonella infection has not been investigated. There is conflicting evidence, as demonstrated in the mouse and calf models, that *spv* genes play a primary role in the establishment and persistence of systemic infections and do not contribute significantly to the enteric phase of the disease. Anaerobiasis was shown to significantly retard the growth rate of *Salmonella* with a significantly reduced cell density at stationary phase, and the *spv* genes were not expressed.[132] This lends further support to the hypothesis that the *spv* genes are not involved in the enteric phase of infection, but this has not been examined in species other than the calf and mouse.

Specific Aims

The overall aim of this section of the study was to describe the general and molecular characteristics of *Salmonella* spp. isolated from hospitalized symptomatic

animals in North Central Florida and contrast these isolates from those collected from asymptomatic animals in the same geographic region. The specific aims were:

- To collect, describe, and store *Salmonella* spp. isolates from hospitalized horses.
- To collect, describe, and store *Salmonella* spp. isolates from asymptomatic horses at pasture.
- To determine if the salmonella isolates carried plasmids and classify them based on size.
- To determine if the salmonella isolates carried *spv* genes.
- To examine plasmid containing *spv* negative isolates for antibiotic resistance determinants located on those plasmids.

Materials and Methods

Case Selection

Bacterial cultures were obtained from hospitalized foals and adult horses with clinical signs consistent with salmonella infection. Material submitted to the clinical microbiology laboratory included feces, gastric secretions, blood, synovial fluid, and tissue samples from post-mortem examinations. Sequential fecal samples were also collected from asymptomatic horses at several farms in the North Central Florida area, over a period of 24 months. Individual records were kept for each animal and horses were sampled at least three times on separate occasions.

Microbiological Techniques

Field samples

Freshly voided or rectal fecal samples were collected and placed into sterile, labeled containers. Two to five grams of fecal material was placed into selenite broth and incubated at 37°C in a 5% CO_2 environment for 12-18 h to maximize isolation of *Salmonella* spp. A Hektoen-Enteric plate (Remel Inc., Lenexa, KS) was streaked for isolation from the overnight culture broth. The plates were incubated 18-24 h at 37°C in a 5% CO_2 environment. Non-lactose-fermenting and H_2S-producing colonies were

selected and streaked onto urease slants (Remel Inc., Lenexa, KS) which were incubated 18-24 h at 37°C in a 5% CO_2 environment. Urease-negative organisms were further characterized utilizing API 20E enteric test strips (bioMérieux USA, Durham, NC) for positive identification of *Salmonella* spp. An incubated strip with reactions typical of *Salmonella* is shown in Figure 2-1. Tests and interpretations from left to right include ONPG -, ADH +, LDC +, ODC +, CIT +, H2S +, URE -, TDA -, IND -, VP -, GEL -, GLU +, MAN +, INO -, SOR +, RHA +, SAC -, MEL +, AMY -, ARA +.

Figure 2-1. API20E rapid identification strip showing typical reaction results for *Salmonella* spp.

Clinical and reference isolates

All clinical isolates were provided as pure cultures on Hektoen-Enteric (HE) agar plates (Figure 2-2) by the clinical microbiology service at the University of Florida College of Veterinary Medicine, Gainesville, Florida.

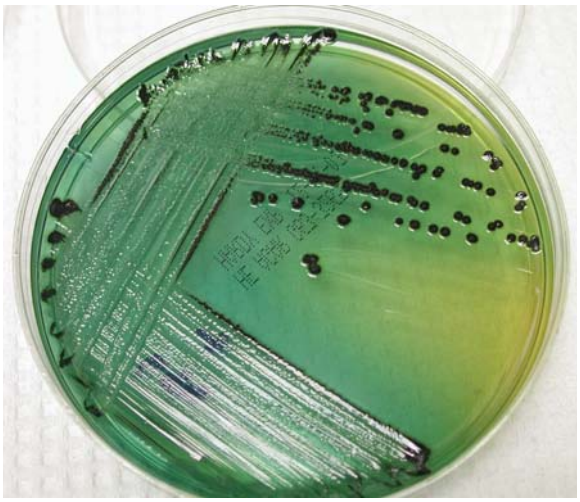

Figure 2-2. Salmonella group C_2 isolate as provided on Hektoen-Enteric agar

Bacterial cultures of all salmonella reference strains and clinical isolates were subsequently grown in Luria-Bertani broth (LB) or on LB agar without antibiotics at 35˚C, in a 5% CO_2 atmosphere unless otherwise indicated. One and a half percent (w/v) agar was added to LB broth for plates. Composition of culture media is in Table 2-2.

Table 2-2. Composition of bacterial culture media

Media	**Ingredients per Liter and/or Supplier with Catalog Number**	**Sterilization**	**Storage**
LB Broth	10 g tryptone 5 g yeast extract 5 g NaCl Sigma-Aldrich L3152	Autoclave 15 min at 121°C	RT
LB Agar	10 g tryptone 5 g yeast extract 5 g NaCl 15 g agar	Autoclave 15 min at 121°C	2-6°C
LB-N Broth	10 g tryptone 5 g yeast extract 8.5 ml 5 M NaCl (0.85% w/v)	Autoclave 15 min at 121°C	RT
Hektoen-Enteric Agar	Sigma-Aldrich H7532	Autoclave 15 min at 121°C	2-6°C
Minimal Medium (M9)	200 ml 5x M9 Salts 20 ml 1 M glucose 2 ml 1 M $MgSO_4$ 0.1 ml 1 M $CaCl_2$	Autoclave 15 min at 121°C **before** addition of filter sterilized glucose	RT or 2-6°C
Brain-Heart Infusion (BHI) Broth	Sigma-Aldrich B7403	Autoclave 15 min at 121°C	RT
Selenite Broth w/ Cystine	Remel 064506	Pre-sterilized commercial product	2-6°C
Urea Agar	Remel 065210	Pre-sterilized commercial product	2-6°C
PRAS Brucella blood-agar with 75 micrograms per ml gentamicin	Anaerobe Systems AS-141G	Pre-sterilized, custom manufactured, commercial product	RT

Antibiotic supplementation to LB agar plates, when referenced, was made in the following concentrations: ampicillin (AMP) 100 micrograms/ml; nalidixic acid (NAL) 50 micrograms/ml; ceftiofur sodium (NAX) 8 micrograms/ml; cefazolin sodium salt (CEF) 8-32 micrograms/ml; and tetracycline hydrochloride (TET) 25 micrograms/ml.

Salmonella Identification and Antibiotic Resistance Profile

Isolates identified as *Salmonella* were grouped using group specific antisera (Fisher Scientific International, Hampton, NH) and serotyped through National Veterinary Service Laboratories (NVSL), Ames, IA. Those isolates positively identified as *Salmonella* were sub-cultured and frozen.

Antibiotic resistance profiles were determined for each isolate via an automated minimum inhibitory concentration (MIC) system (Sensititre® Microbiology Systems, software version—SAMS V2.3 Release 1, Trek Diagnostics, Cleveland, OH, USA).

Salmonella Isolate Storage

Subcultures of salmonella strains were stored as pure cultures at -80°C in LB or Brain-Heart Infusion (BHI) and 35% (v/v) glycerol. A standing overnight culture was prepared by selecting approximately ten to fifteen colonies from the plate provided by the microbiology laboratory. Multiple colonies were sampled to avoid the selection of any single genotype or abnormal colony. The isolate was inoculated into LB and incubated at 37˚C without agitation. The following morning, 1 ml of this culture was added to 30 ml of either BHI or LB and incubated at 37˚C with agitation for 1 to 1.5 hours (approximate OD600 = 0.5-0.6). The sample was centrifuged at 10,000 x *g* for 10 min to pellet the cells. The supernatant was removed, and the cells were re-suspended into 2 ml BHI or LB. Two ml of 70% glycerol was added to the cell suspension and mixed gently. The

isolates were transferred immediately to pre-labeled standard cryogenic storage vials and flash frozen in a dry ice and ethanol bath.

Reference Strains

Reference *Salmonella* serovar Typhimurium strains χ3306 and χ3337 were kindly provided by Dr. Paul Gulig in the Department of Molecular Genetics and Microbiology at the University of Florida College of Medicine. Specific information regarding these strains is detailed in Table 2-3. The sequenced size of the *S*. Typhimurium strain LT2 virulence plasmid pSLT is 93,939 bp,[133] but the virulence plasmid sizes of similar strains such as SR-11 may vary. The reported size for the virulence plasmid of the strain used herein is approximately 100,000 bp.

Table 2-3. *Salmonella* serovar Typhimurium reference strains used in this study

Strain	Genotype	Source	Phenotype
SR-11 χ3306	*gyrA1816*, pStSR100^{+}	Dr. Paul Gulig	Nal^{r}, virulent, *spv*$^{+}$
SR-11 χ3337	*gyrA1816*, pStSR100^{-}	Dr. Paul Gulig	Nal^{r}, *spv*$^{-}$, avirulent, plasmid cured derivative of χ3306

Plasmid Profiling of Salmonella Isolates

Plasmid extraction was achieved using a modification of a commercial kit for large construct and very low copy number plasmid purification (Qiagen Filter Midi Kit, Qiagen, Inc., Valencia, CA). The *S.* Typhimurium plasmid copy number has been estimated to be between 2-3 per cell.[133] Bacteria were grown in 50 ml LB for 12-16 h (approximately $A_{600} = 1 – 1.5$). Cells were divided into two sterile 50 ml polypropylene centrifuge tubes and pelleted by centrifugation at 7,000 rpm in JA-20 rotor for 15 min at 4°C. The cells were re-suspended thoroughly by vortexing in 10 ml buffer P1-Resuspension Buffer per tube. Ten ml of buffer P2-Lysis Buffer was added to each tube, the cells were mixed by gentle rolling and inversion and incubated at RT for 5 min

exactly. Ten ml of chilled buffer P3-Neutralization Buffer was added per tube and the samples were mixed immediately but gently by inversion. The tubes were incubated for 15 min at RT. Columns (Qiagen-tip 100, Qiagen Inc., Valencia, CA) were equilibrated with 4 ml of buffer QBT and columns were allowed to empty by gravity flow during this incubation to be ready when needed. Samples were poured into pre-labeled high-speed centrifuge tubes (Oak Ridge Centrifuge Tubes, Fisher Scientific International, Hampton, NH) and centrifuged at 15,000 rpm in a Beckman JA-20 rotor, for 10 min at 4°C. The supernatant was removed and applied to a vertically supported filtration syringe (QIAfilter cartridge, Qiagen Inc., Valencia, CA), and the plunger was inserted. The filtrate was dispensed onto the columns gently and slowly, over a period of approximately 10-20 min, keeping visible sample in the reservoir of the column at all times. The column was then washed with 2 volumes of 10 ml buffer QC-Wash Buffer at RT and allowed to empty by gravity flow. The wash solutions were discarded and clean 40 ml high-speed centrifuge tubes were placed under the columns to collect the eluted DNA. Plasmid DNA was eluted with 5 ml 56°C buffer QF-Elution Buffer per column. The plasmid DNA was precipitated with 0.7 volumes (4 ml per isolate) of RT isopropanol. The samples were centrifuged at 16,000 rpm in a JA-20 rotor for 30 min at 4°C and the supernatant was removed. The DNA pellet was washed with 70% ethanol, dried, and re-suspended in 150 microliters of TE for agarose gel analysis and transformation experiments. A summary of the solution ingredients for the modified plasmid extraction procedures and storage is found in Table 2-4.

Agarose gel analysis was performed via common method. Equal volumes of the plasmid extract and 10x sample loading buffer were combined as a droplet on paraffin

paper, loaded, and run on a 0.5% agarose in Tris-borate-EDTA (TBE) gel at 125 volts for 1.5 h. Size was estimated by comparison to the approx. 100-kb plasmid from *S.* Typhimurium χ3306 run on the same gel in addition to a super coiled DNA ladder with a range from 16.2 to 2-kb pairs (Gibco BRL, Carlsbad, CA). Plasmid bands were visualized by staining the gel with ethidium bromide (1 microgram/ml) and photographed using a digital gel imaging and documenting system (Chemi System, UVP BioImaging Systems, Upland, CA).

If an isolate had at least one large plasmid it was considered plasmid-positive. A plasmid was only considered to be a virulence plasmid if the *spv* gene primer sets hybridized to the isolate. Otherwise, it was simply a large plasmid of unknown type.

Table 2-4. Composition of buffers and solutions used in plasmid extraction protocols

Reagent	Composition	Storage
P1 (Resuspension Buffer)	50mM Tris-HCl pH 8.0 10mM EDTA 100 micrograms/ml RNase A	2-8°C
P2 (Lysis Buffer)	200mM NaOH 1% SDS (w/v)	RT
P3 (Neutralization Buffer)	3.0M potassium acetate pH 5.5	RT or 2-8°C
QBT (Equilibration Buffer)	750mM NaCl 50mM MOPS pH 7.0 15% isopropanol (v/v) 0.15% Triton® X-100 (v/v)	RT
QC (Wash Buffer)	1.0M NaCl 50mM MOPS pH 7.0 15% isopropanol (v/v)	RT
QF (Elution Buffer)	1.25M NaCl 50mM Tris-HCl pH 8.5 15% isopropanol (v/v)	RT
Tris-EDTA (TE)	10mM Tris-HCl pH 8.0 1mM EDTA	RT
10x Sample Loading Buffer	40% Sucrose 0.17% Xylene Cyanol 0.17% Bromophenol Blue	RT

Polymerase Chain Reaction (PCR) Identification of *spv* Genes

PCR was performed to evaluate all clinical isolates for the presence of the *spv* genes. Positive (χ3306) and negative (χ3337) isolates, as well as a series for a chromosomal gene, aspartate semialdehyde dehydrogenase (*asd*), were run as controls in each experiment. These controls were vital for two reasons: 1) to verify that the reactions, reagents, and conditions were appropriate, and 2) to ensure that the isolates were truly *Salmonella* spp. (which was most important in validating negative reactions). A loop of pure culture was added to 200 microliters of sterile water in a 1.5 ml microcentrifuge tube, and boiled for 10 min to be used as template DNA. The master mix and all reactions were prepared and maintained on ice until the run was started. Master mix was made fresh for each experimental run, and consisted of 24.75 microliters deionized H_20, 5 microliters 10x PCR buffer, 8 microliters 1.25mM dNTP mix, 0.25 microliters 5U/microliter *Taq* DNA polymerase, and 4 microliters 50mM $MgCl_2$ (GibcoBRL, Carlsbad, CA). Five microliters of template DNA was added to each tube for a total reaction volume of 50 microliters per tube. The samples were placed in a thermocycler (Programmable Thermal Controller PTC-100®, MJ Research, Inc. Reno, NV) for the cycle described in Table 2-5.

Table 2-5. Times and temperatures for PCR reactions

STEP 1	Melt	94°C	180 seconds
STEP 2	Melt	94°C	60 seconds
STEP 3	Anneal	45°C or 50°C	60 seconds
STEP 4	Extend	72°C	120 seconds
REPEAT STEPS 2, 3, & 4 FOR 30 CYCLES			
STEP 5	End	72°C	180 seconds
STEP 6	Hold	4°C	indefinitely

Primer sets for the *spv* gene PCR were provided by Dr. Paul Gulig. These consisted of 3' and 5' primers for *asd*, *spvA*, *spvC*, and *spvR*. All clinical isolates were

examined for presence of the *asd* gene of *Salmonella* and probed with at least two different *spv* gene primer sets. An isolate was determined to be positive for the *spv* gene locus if two conditions were met: 1) if the *asd* product was present, and 2) if two or more of the *spv* gene products were present. These primer sets are situated to bracket the entire open reading frame of the gene, so the PCR products are in essence whole *spv* genes. All isolates were probed with no less than two *spv* gene primer sets each (usually *spvA* and *spvC*, occasionally including *spvR*). These primers were extremely effective in their ability to identify the genus *Salmonella* and the presence of *spv* genes. Sequence and other important primer information are contained in Table 2-6.

Table 2-6. Primers utilized in PCR reactions

Primer	**Sequence**	**Product Size**
spvA 5’	5’-CCCCCGGGATGAATATGAATCAGACCACCA-3’	---
spvA 3’	5’-GGGAATTCTGGTAGCGCGGGAAGC-3’	≈784 bp
asd 5’	5’-CAGCACATCTCTTAGCAGGAAAAAAACGC-3’	---
asd 3’	5’-GGGAAGCTTCTACGCCAACTGGCGCA-3’	≈1,100 bp
spvR 5’	5’-CCCCGGGATCCATGGATTTCTTGATTAATAAA-3’	---
spvR 3’	5’-CCCCGGGAATTCGCTGCATAAGGTCAGAAGG-3’	≈905 bp
spvC 5’	5’-CCCCCGGGATGCCCATAAATAGGCCTAATC-3’	---
spvC 3’	5’-GCCGGAATTCGTCAGTAAGGG-3’	≈875 bp

Salmonella Plasmid Transformations into Susceptible Bacteria—Effects on Antibiotic Resistance

Based on a discovery that the minority of large plasmids in the clinical salmonella isolates were virulence plasmids, transformations of extracted plasmid DNA into a select antibiotic-sensitive strain of *Escherichia coli* were performed to investigate the possibility that the large plasmids may be carrying antibiotic resistance (R) determinants.

Three clinical salmonella isolates were selected based on their antimicrobial sensitivity profiles and the presence of a single large plasmid that did not contain the *spv* genes. Successful transference was confirmed through plasmid extraction of the

transformed *E. coli* isolates and gel electrophoresis with untransformed *E. coli* as well as the original plasmid extracts used for the transformation. Briefly, the procedure was performed as follows: competent *E. coli* DH5α cells (Invitrogen Corporation, Carlsbad, CA) were thawed on ice. A 40 microliter aliquot of *E. coli* was added to an ice-cold electroporation cuvette. All solutions were maintained on ice throughout the procedure unless otherwise specified. Two microliters of plasmid extract in TE was added to the *E. coli* and mixed gently with a pipette. The mixture was electroporated at 1.25 kV, 25 microfarad capacitance 200 ohms resistance, on a Bio-Rad Gene-Pulser (Bio-Rad, Hercules, CA). The time constant was as close to 4.9 as possible, and if below 4.5, the procedure was repeated with less plasmid extract. Nine-hundred microliters LBN broth was added to the cuvette and it was incubated in a water bath at 37°C for 30 minutes. The cell suspension was transferred to a 1.5 ml microcentrifuge tube and 100 microliters was spread onto several different LB agar plates, each containing a relevant selective antibiotic based on the antimicrobial resistance profile of the original salmonella isolate. The plates were incubated overnight at 37°C and observed for growth the next day. Transformants (as evidenced by growth on selective plates) were grown in LB broth with continued selective pressure and subjected to the same extraction procedure described previously to verify the presence and size of newly acquired plasmid DNA.

Chemical transformations were performed by mixing 5 microliters of extracted donor plasmid DNA in water with 40 microliters of recipient strain (same DH5α *E. coli* as for electroporation transformations). The mixture was incubated on ice for 15 min and then transferred to 42˚C water bath for 2 min to heat-shock the cells. Five-hundred microliters of LBN broth was added, and the mixture was incubated at 37˚C without

shaking for 30 min. One-hundred microliters of the mixture was then plated on appropriate selective plates as described for the electroporation transformation.

Statistical Methods

Clinical isolates, patient information, and resistance data were collected weekly as they became available over a period of three years (December 1999 through September 2002). Survival was based on discharge from the hospital. Animals were initially grouped with respect to age into the following categories: <0.5y; 0.5-4y; 5-8y; 9-12y; 13-15y; and >15y. They were then further grouped as follows for statistical comparison: 0-5y; 6-15y; and >15y. Cases with missing data were excluded from calculation of descriptive percentages (e.g., survival, gender, age). The effects of gender, age, and breed on outcome were investigated independently using the Pearson Chi-square test. Stepwise logistic regression was used to form a model in which multiple clinical variables could be used to predict outcome. A Kruskal-Wallis test was used to investigate the percentage of submitted samples that were positive with respect to outcome.

Results

Asymptomatic Population

Isolates were sought from asymptomatic animals in order to contrast bacterial genotype with isolates from clinical cases. *Salmonella* spp. were not identified from any of 381 cultures performed on 105 different asymptomatic horses over a period of two years.

Clinical Cases

There were 106 hospitalized animals during the period of interest that had at least one positive culture of *Salmonella*. Within this population there were more males (61%)

than females (39%), although this difference was not significant. The mean age of the population was 4.9 years, with a range of 2 days to 38 years. The age distribution by categorical group is shown in Figure 2-3. Fifty seven percent of the clinical cases survived. Of those non-survivors 26% died spontaneously. Breed distribution for the affected horses is shown in Table 2-7.

Table 2-7. Breed distribution of 84 equine salmonella cases 1999-2002

Breed	No. of Cases	% of Cases
Thoroughbred	32	38.09
Quarter Horse	15	17.86
Paso Fino	10	11.90
Miniature Horse	5	5.95
Arabian	4	4.76
Paint Horse	4	4.76
Warmblood	4	4.76
Standardbred	3	3.57
Pony	3	3.57
Mixed Breed	2	2.38
Draft	1	1.19
Appaloosa	1	1.19

Relationship Between Gender or Age and Outcome

There was no gender bias with respect to short-term survival (Table 2-8). There was a statistically significant association between age and case outcome (Table 2-9). Horses less than 5 years of age were 3.3 times more likely to die when infected with *Salmonella* than older animals.

Table 2-8. Effect of gender on mortality in 96 cases of equine salmonellosis*

Gender	Died	Survived	% Dead	Odds Ratio	Lower 95%CI	Upper 95%CI
Female	13	19	40.6	1.25	0.54	2.88
Male	22	30	42.3	1.00	0.47	2.13

*Pearson Chi-square value of 0.277 with 1 degree of freedom, p=0.599

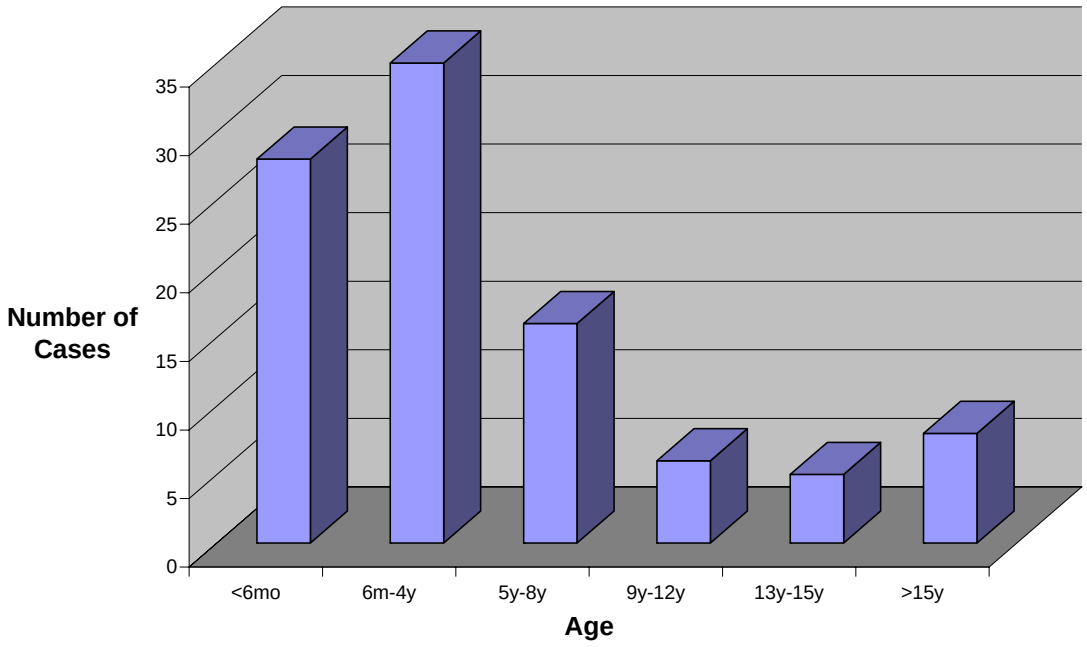

Figure 2-3. Age distribution of 98 equine salmonella cases 1999-2002

Table 2-9. Effect of age on mortality in 85 cases of equine salmonellosis*

Age Group	Died	Lived	% Dead	Odds Ratio	Lower 95%CI	Upper 95%CI
0 – 4 yrs	28	28	50.00%	3.33	1.18	9.42
5 – 15 yrs	6	17	26.09%	1.00	0.28	3.63
> 15 yrs	2	4	33.33%	1.67	0.24	11.45

* Pearson Chi-square value of 6.002 with 2 degrees of freedom, p = 0.05.

Case Seasonality

Cases were examined for the month of occurrence and the data are shown in Figure 2-4. The majority of cases in the present study occurred during the warmer months of the year, with 68% between the months of April and September. Thirty-year average minimum temperatures in Gainesville, Florida remained above 60.3˚F (15.7˚C) during the months of May through October (Table 2-10).

Table 2-10. Average minimum temperatures in Gainesville, Florida, USA (1961-1990)*

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year Avg
°C	6.1	6.8	10.2	13.1	17.1	20.6	21.7	21.8	20.7	15.7	10.9	7.4	**14.3**
°F	43.0	44.2	50.4	55.6	62.8	69.1	71.1	71.2	69.3	60.3	51.6	45.3	**57.7**

*Obtained from www.worldclimate.com

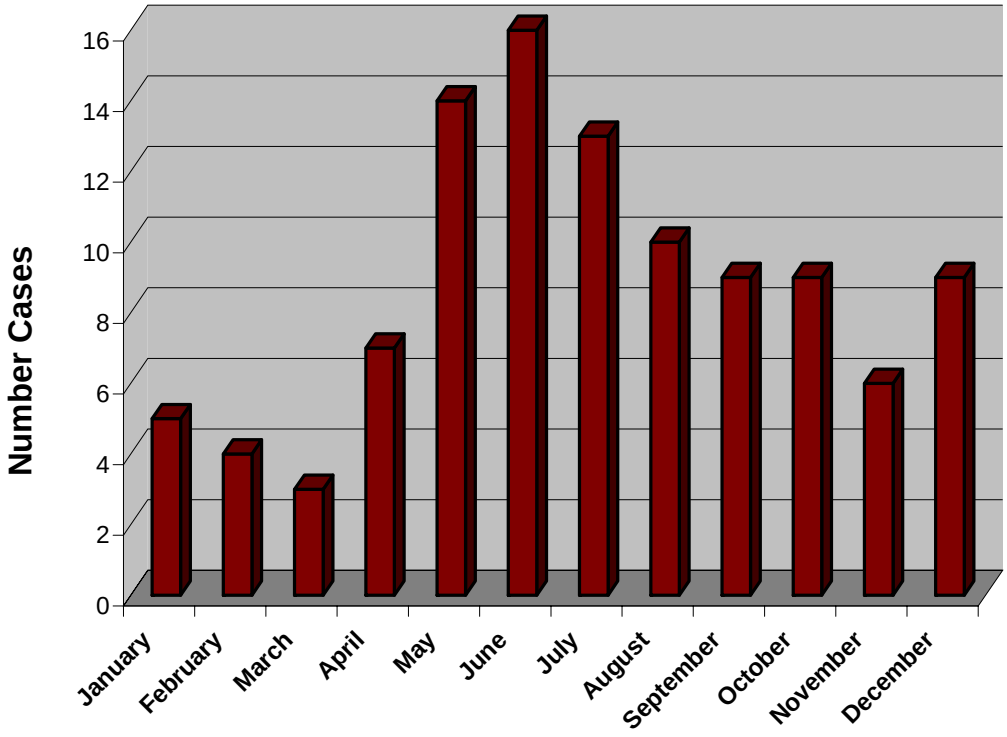

Figure 2-4. Seasonal distribution of salmonella cases from horses 1999-2002

Group and Serovar Distribution

All serovars in the present study have been previously identified in horses with the exception of a group F *Salmonella* serotyped as Rubislaw. There were three cases serotyped as *Salmonella* 4,5,12:i—monophasic, a type closely related to *S*. Typhimurium, whose antigenic formula is 1,4,5,12:i—1,2 biphasic.[134] Eight isolates (7.5%) were not serotyped as the samples were either contaminated with other bacterial genera or contained more than one group or serovar of *Salmonella*. The breakdown of salmonella group, serovar, and prevalence in this study is summarized in Table 2-11.

Forty-eight additional isolates from environmental sampling and animal species other than horses were collected and archived during the reporting period. These isolates were serotyped, and antibiotic sensitivity profiles determined, but no other analyses were performed. Serovar and species of isolation information regarding these isolates is detailed in Table 2-12.

Table 2-11. Salmonella serovars isolated from 98 equine cases 1999-2002

Serovar	**Group**	**Number of Cases**	**% of Isolates**
Java	B	23	23.45
Newport	C2	13	13.27
Typhimurium	B	8	8.16
Typhimurium var. Copenhagen	B	7	7.14
Javiana	D	7	7.14
Miami	D	7	7.14
Saintpaul	B	6	6.12
Muenchen	C2	5	5.10
Anatum	E	4	4.08
4,5,12:i-monophasic	B	3	3.06
Newington	E	2	2.04
London	E	2	2.04
Mbandaka	C1	2	2.04
Hartford	C1	1	1.02
Agona	B	1	1.02
Braenderup	C1	1	1.02
Infantis	C1	1	1.02
Meleagridis	E	1	1.02
Reading	B	1	1.02
Rubislaw	F	1	1.02
Tallahassee	C2	1	1.02
Thompson	C1	1	1.02

Table 2-12. Salmonella isolates of environmental and species other than equids collected 1999-2002

Isolate Origin	**Serovar**	**Group**	**No. of Isolates**
Avian	Manila	E	1
Avian	Infantis	C1	1
Bovine	Typhimurium	B	4
Bovine	Typhimurium var. Copenhagen	B	4
Bovine	Anatum	E	2
Bovine	Newport	C2	1

Table 2-12. Continued

Isolate Origin	Serovar	Group	No. of Isolates
Bovine	Mbandaka	C1	1
Canine	Typhimurium var. Copenhagen	B	2
Canine	Adelaide	Not A-E	2
Canine	Miami	D	1
Environmental	Java	B	15
Environmental	Newport	C2	3
Environmental	Typhimurium var. Copenhagen	B	2
Environmental	Javiana	D	1
Environmental	Anatum	E	1
Environmental	Typhimurium	B	1
Environmental	Tallahassee	C2	1
Non-human Primate	Typhimurium	B	1
Other Mammal	Hartford	C1	1
Reptile	Sub group 3		1
Rodent	Typhimurium var. Copenhagen	B	1

Outcome by Group or Serovar

The relationship between group or serovar and outcome was investigated. The results are presented graphically in Figure 2-5. There was a significant difference between isolates according to antigenic grouping in terms of mortality (p=0.033; Figure 2-6); survival was decreased with isolation of group B salmonella serovars (43% survival). Other groups included C1 (60% survival), C2 (59% survival), D (92% survival), E (67% survival), and F (100% survival). Odds ratio data were determined by salmonella group; if the horse was infected with a group B *Salmonella*, it was 15.7 times more likely to die (1.9 to 129.25, 95%CI) than if it were infected with a group D. Statistical summary of data is shown in Table 2-13. Additional odds ratios, relative to infection with group D, were: C1—6 times more likely to die; C2—7 times more likely to die; and E—6 times more likely to die. Although these ratios appeared large they were not significant as the respective confidence intervals included 1.0.

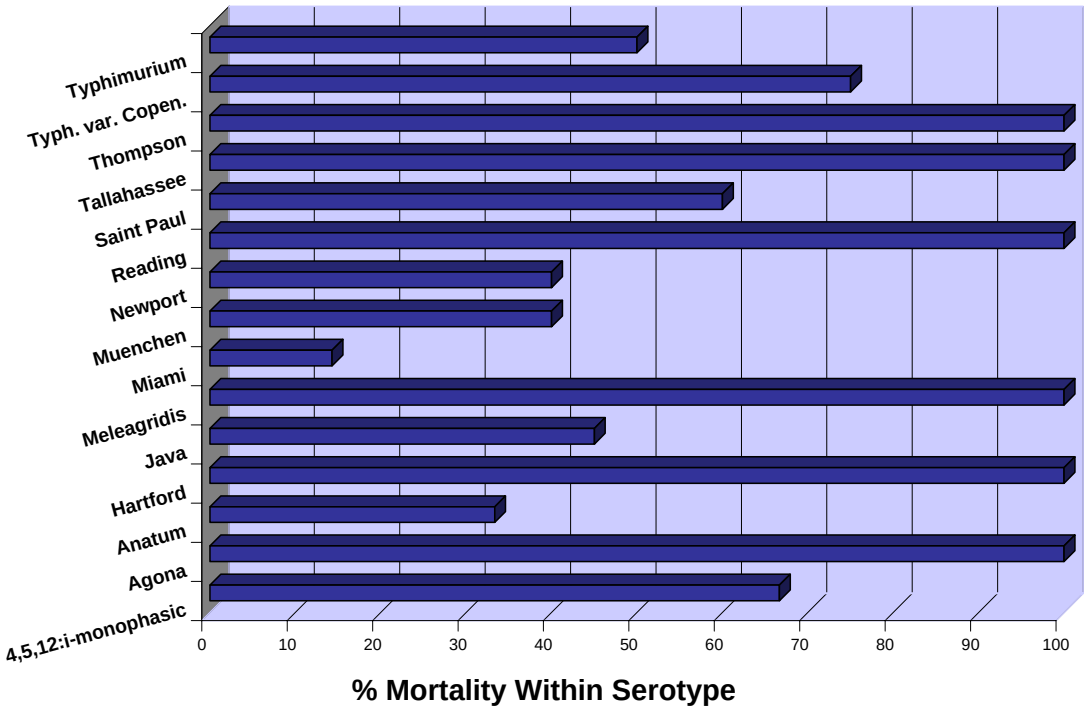

Figure 2-5. Mortality distribution, within serovar, of non-surviving equine salmonella cases 1999-2002

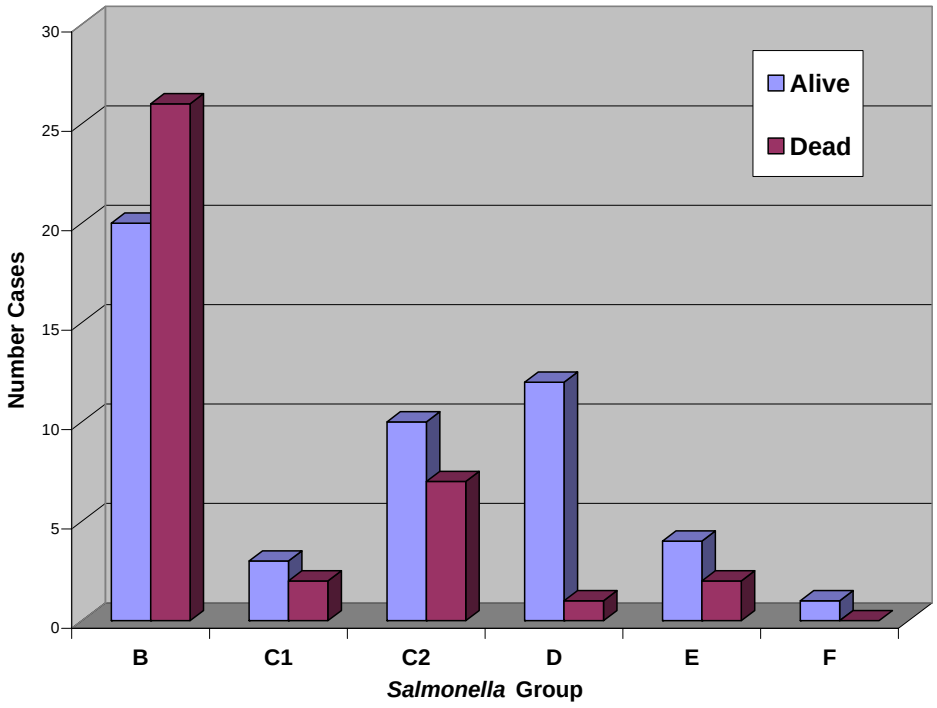

Figure 2-6. Mortality by salmonella group in 88 cases with known outcomes

Table 2-13. Effect of salmonella group on mortality in 88 cases of equine salmonellosis*

Group	Died	Lived	% Dead	Odds Ratio	Lower 95%CI	Upper 95%CI
B	26	20	56.5	15.7	1.90	129.25
C1	2	3	40.0	6.0	0.42	85.25
C2	7	10	41.2	7.0	0.74	65.95
E	2	4	33.3	6.0	0.42	85.25
D	1	12	7.7	1.0	0.06	17.90
F	0	1	0.00	----	----	----

* Pearson Chi-square value of 12.129 with 5 degrees of freedom, p = 0.033.

Within group B organisms, *S.* Typhimurium was associated with the highest mortality rate (75.0% mortality), followed by 4,5,12:i-monophasic (66.7% mortality), *S.* Saintpaul (60.0% mortality), *S.* Typhimurium var. Copenhagen (50.0% mortality), and *S.* Java (45.0% mortality). Four serovars (Tallahassee, Reading, Meleagridis, and Agona) may appear more virulent due to low numbers of cases.

Plasmid Profiling

Plasmid profiles were completed for 104 clinical salmonella isolates. Several isolates in the main database were not analyzed due to equivocal identification, inability to culture the provided isolate sample, or loss during storage. The majority of examined isolates, 64.4% (67/104), contained at least one large (> 20-kb) plasmid. Several isolates had additional smaller plasmids and some had more than one large plasmid. The breakdown of plasmid carriage by serovar is listed in Table 2-14.

Table 2-14. Plasmid-positive salmonella isolates by serovar 1999-2002

Serovar	Number Plasmid Positive / Number Serovar	% Positive of Total Isolates	% Positive Within Serovar
Java	23 / 25	22.12	92.00
Newport	5 / 13	4.81	38.46
Typhimurium	8 / 8	7.69	100.00
Typhimurium var. Copenhagen	7 / 8	6.73	87.50
Javiana	3 / 5	2.88	60.00
Miami	1 / 7	0.96	14.29

Table 2-14. Continued

Serovar	Number Plasmid Positive / Number Serovar	% Positive of Total Isolates	% Positive Within Serovar
Muenchen	6 / 6	5.77	100.00
Saintpaul	2 / 6	1.92	33.33
Unidentified	2 / 4	1.92	50.00
Anatum	2 / 4	1.92	50.00
4,5,12:i-monophasic	3 / 3	2.88	100.00
Newington	0 / 2	0.00	0.00
Hartford	2 / 2	1.92	100.00
London	1 / 2	0.96	50.00
Mbandaka	1 / 2	0.96	50.00
Agona	0 / 1	0.00	0.00
Braenderup	0 / 1	0.00	0.00
Infantis	0 / 1	0.00	0.00
Meleagridis	0 / 1	0.00	0.00
Reading	0 / 1	0.00	0.00
Rubislaw	1 / 1	0.96	100.00
Tallahassee	0 / 1	0.00	0.00
TOTAL	**67 / 104**	**64.42%**	

Selected examples of results from agarose gel electrophoresis plasmid profiles are shown in Figures 2-7 through 2-13. Figure 2-7 shows the plasmid profiles of several isolates extracted using the Birnboim and Doly method.[135] All isolates were considered plasmid-positive; however, lanes 3, 5, 6, and 7 show plasmids that were slightly larger than the 100-kb plasmid of χ3306. Three of those four were *S*. Typhimurium var. Copenhagen isolates of bovine origin, and the fourth was an equine *S*. Newport. Figure 2-8 shows the plasmid profiles of Case 71 in lane 4, Case 66 in lane 5, and Case 77 in lane 6. All of these isolates were determined to be plasmid-negative. These isolates were identified as *S*. Miami, *S*. Newport, and *S*. Miami respectively. Figure 2-9 shows the plasmid profile of five cases which all demonstrated large (> 16-kb) plasmids, but varying in size relative to the 100-kb plasmid of the control strain. Only Case 89 (identified as *S*. Typhimurium var. Copenhagen) possessed the *spv* genes—all others

were negative. Interestingly, that plasmid appears very close in size to the 100-kb virulence plasmid of the control *S*. Typhimurium strain, while the other four isolate plasmids are large, but not necessarily equivalent in size. Figure 2-10 shows the plasmid profile of Case 44 in lane 5 and Case 43 in lane 6. Both isolates appeared to possess a single large (≈ 100-kb) and a single small plasmid. Both of these isolates were identified as *S*. Typhimurium var. Copenhagen from horses having antibiotic administration prior to admission, and neither of these isolates possessed the *spv* genes. Figure 2-11 shows the plasmid profile of Case 63 in lane 5. Case 63 appeared to possess multiple plasmids ranging in size from approximately 2-kb to > 100-kb but did not possess the *spv* genes. This isolate was identified as *S*. London from a horse with prior antibiotic administration (penicillin G). Interestingly, this isolate was not considered to be one of the more multi-drug resistant strains to the 19 antimicrobials tested (resistance to more than 13/19 typical in multi-drug resistant strains). The isolate from Case 63 was resistant to clindamycin, doxycycline, erythromycin, oxacillin, penicillin, rifampin, and tetracycline. Figure 2-12 shows the plasmid profile of Case 83 in lane 5 and Case 40 in lane 6. Case 83 did not appear to have any plasmid of any size visible on the gel, while Case 40 appeared to possess both a small and large plasmid (between 4-kb and > 100-kb respectively). Case 83 was identified as *S.* Reading from a necropsy large intestine specimen with unknown cause of death. Case 40 was identified as *S.* Typhimurium var. Copenhagen from a horse with prior antibiotic administration (trimethoprim-sulfamethoxazole, metronidazole and penicillin), and this was one of the few isolates of this serovar to not possess the *spv* genes. Figure 2-13 shows the plasmid profile of Case 36 in lane 3. This isolate was serotyped as the only group F *Salmonella* identified in horses (*S*. Rubislaw). Group F

salmonellae have not been reported as common equid isolates in the literature. This isolate appeared to possess two large and two small plasmids but did not carry the *spv* genes and was obtained from 3/5 fecal samples submitted.

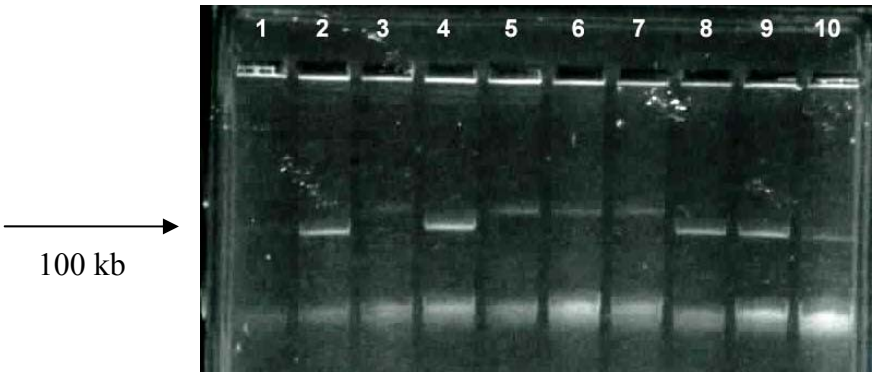

Figure 2-7. Plasmid profiles of 9 clinical salmonella isolates. Refer to Appendix C for specific isolate information. Lanes: 1) Previously extracted 100-kb plasmid of χ3306, 2) Case 8, 3) Bovine isolate of *S*. Typhimurium var. Copenhagen, 4) Case 11, 5) Bovine isolate of *S*. Typhimurium var. Copenhagen, 6) Case 12, 7) Bovine isolate of *S*. Typhimurium var. Copenhagen, 8) Case 6, 9) Case 10, 10) Case 7.

spv Gene Analysis

Of the 67 isolates found to be plasmid-positive 19.4% (12.5% of all isolates) were also PCR-positive for the *spv* genes examined. All positive isolates generated expected PCR product for all genes examined, they were an "all or none" result. Also, all isolates that were *spv* positive were also plasmid-positive, and it was assumed that the genes were located on a plasmid. Figures 2-14 through 2-18 show examples of the results obtained for the *spv* gene analyses of clinical isolates. All isolates positive for the *spv* genes were exclusively group B salmonellae. Within this group they were also limited to only three serovars, Typhimurium, Typhimurium var. Copenhagen, and 4,5,12:i-monophasic, an antigenically close relation to *S.* Typhimurium.

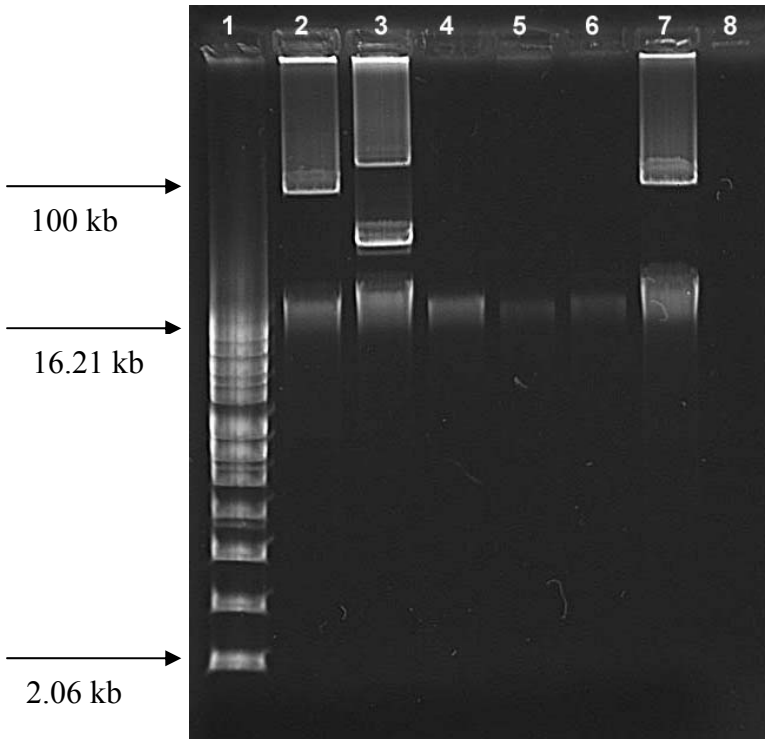

Figure 2-8. Plasmid profiles of 4 clinical salmonella isolates. Refer to Appendix C for specific isolate information. Lanes: 1) supercoiled marker DNA, 2) 100-kb plasmid of χ3306, 3) Case 78, 4) Case 71, 5) Case 66, 6) Case 77, 7) 100-kb plasmid of χ3306, 8) blank.

In Figure 2-14, there are multiple background bands in the *spvA* gel as well as one band in the negative control lane of the *spvC* gel. Since none of these bands were of the same intensity as the control, nor were they an appropriate size, they were considered artifacts. No clinical isolate tested in Figure 2-14 was considered positive for the *spv* genes. There appears to be a faint band of appropriate size in lane 9 of the *spvC* gel; however, since the product band was of low intensity (compared to the positive control) and there was no corresponding positive result in the *spvA* or *spvR* (not shown) gels, the isolate was determined to be negative.

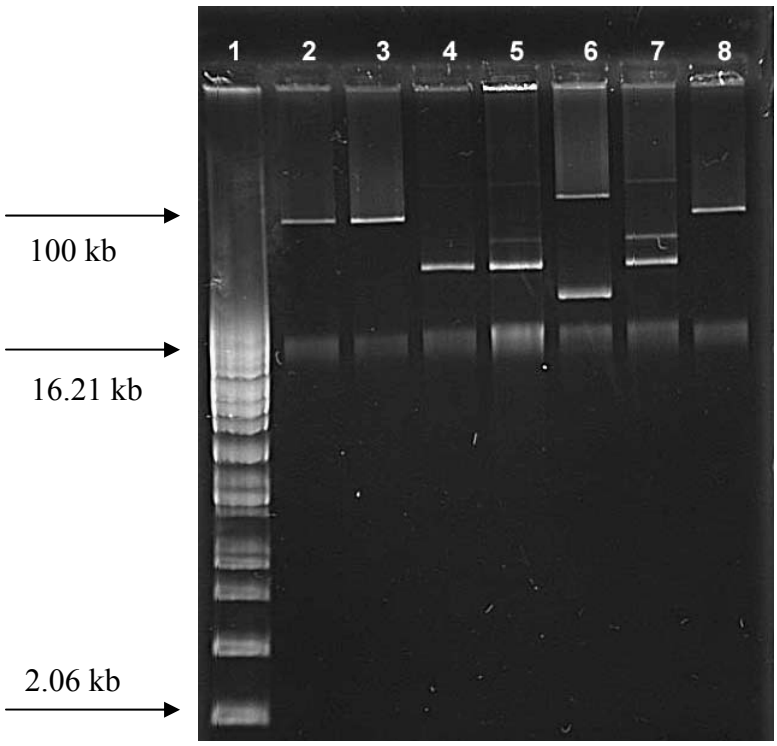

Figure 2-9. Plasmid profiles of 5 clinical salmonella isolates. Refer to Appendix C for specific isolate information. Lanes: 1) supercoiled marker DNA, 2) 100-kb plasmid of χ3306, 3) Case 89, 4) Case 92, 5) Case 85, 6) Case 93, 7) Case 96, 8) 100-kb plasmid of χ3306.

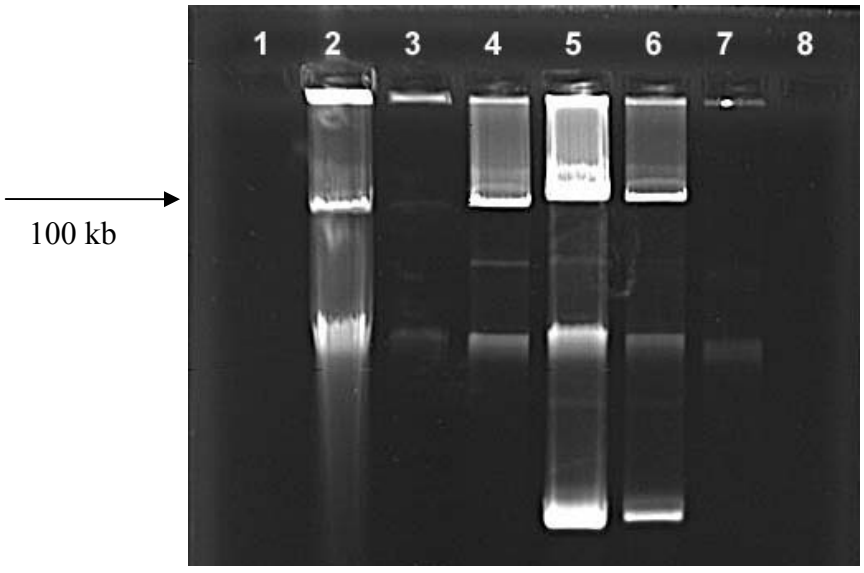

Figure 2-10. Plasmid profiles of 4 clinical salmonella isolates. Refer to Appendix C for specific isolate information. Lanes: 1) blank, 2) 100-kb plasmid of χ3306, 3) Aged (>1month) plasmid extract of χ3306, 4) Case 46, 5) Case 44, 6) Case 43, 7) Case 53, 8) blank.

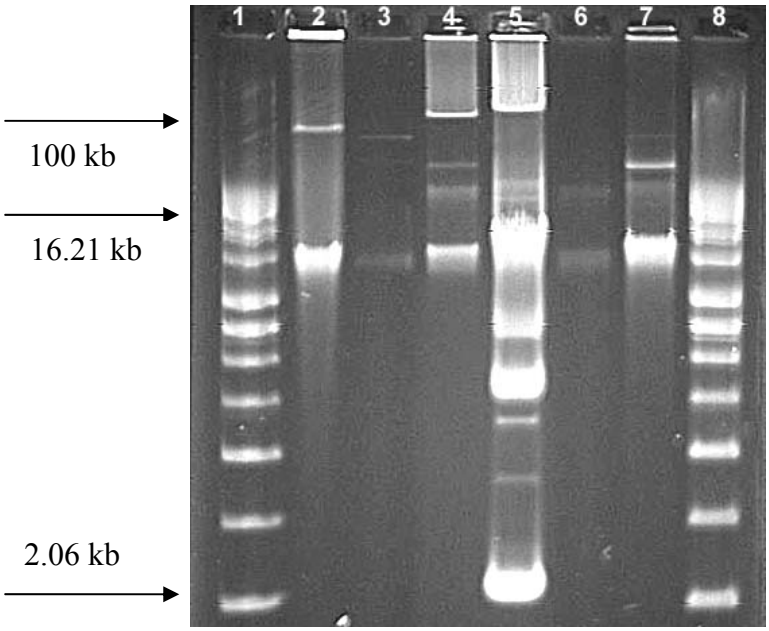

Figure 2-11. Plasmid profiles of 4 clinical salmonella isolates. Refer to Appendix C for specific isolate information. Lanes: 1) supercoiled marker DNA, 2) 100-kb plasmid of χ3306, 3) Aged (>2month) plasmid extract of χ3306, 4) Case 41, 5) Case 63, 6) Case 64, 7) Case 65, 8) supercoiled marker DNA.

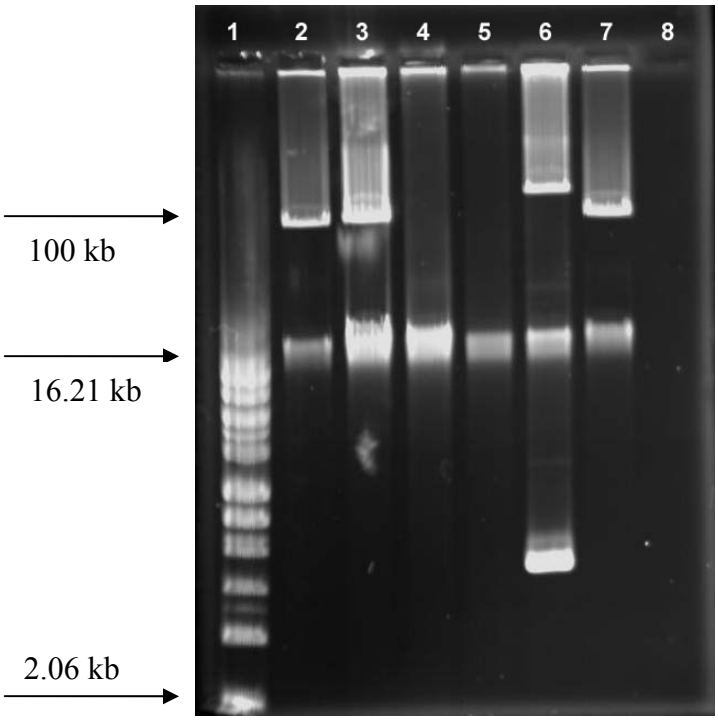

Figure 2-12. Plasmid profiles of 3 clinical salmonella isolates. Refer to Appendix C for specific isolate information. Lanes: 1) supercoiled marker DNA, 2) 100-kb plasmid of χ3306, 3) 100-kb plasmid of χ3306, 4) Case 82, 5) Case 83, 6) Case 40, 7) 100-kb plasmid of χ3306, 8) blank.

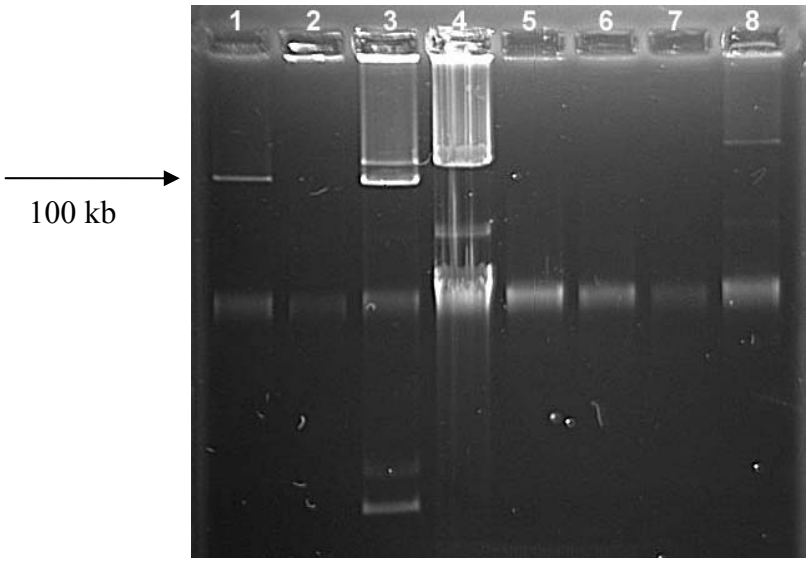

Figure 2-13. Plasmid profiles of 7 clinical salmonella isolates. Refer to Appendix C for specific isolate information. Lanes: 1) 100-kb plasmid of χ3306, 2) Case 32, 3) Case 36, 4) Case 37, 5) Case 91, 6) Case 90, 7) Case 87, 8) Case 86.

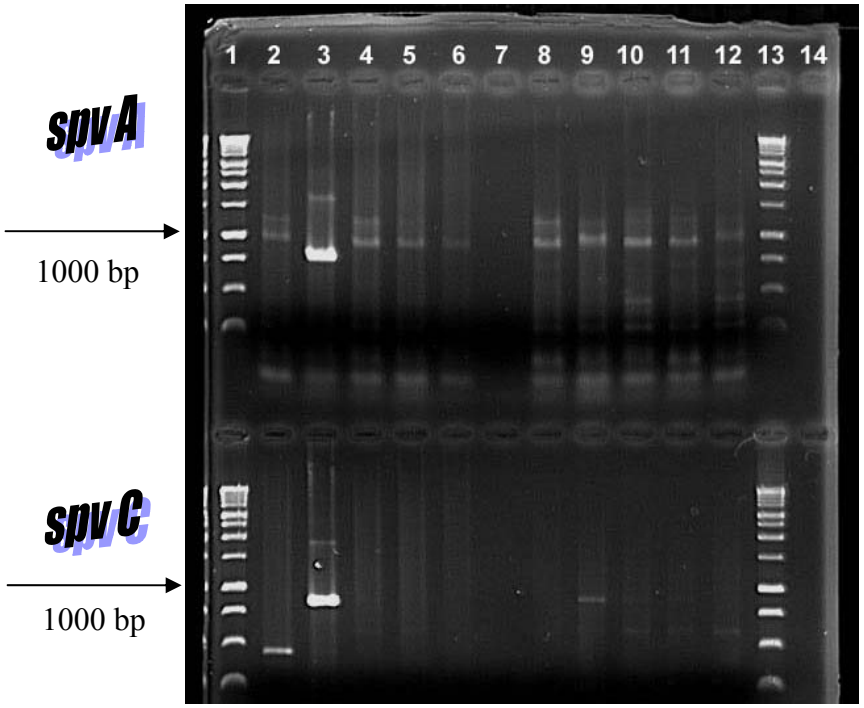

Figure 2-14. PCR product results for *spvA* and *spvC* genes in 9 clinical salmonella isolates, with positive and negative controls. Refer to Appendix C for specific isolate information. Lanes: 1) 1-kb ladder DNA marker (Promega), 2) χ3337 *spv* negative control, 3) χ3306 *spv* positive control, 4) Case 86, 5) Case 87, 6) Case 88, 7) Case 117, 8) Case 100, 9) Case 101, 10) Case 102, 11) Case 103, 12) Case 104, 13) 1-kb ladder DNA marker (Promega), 14) blank.

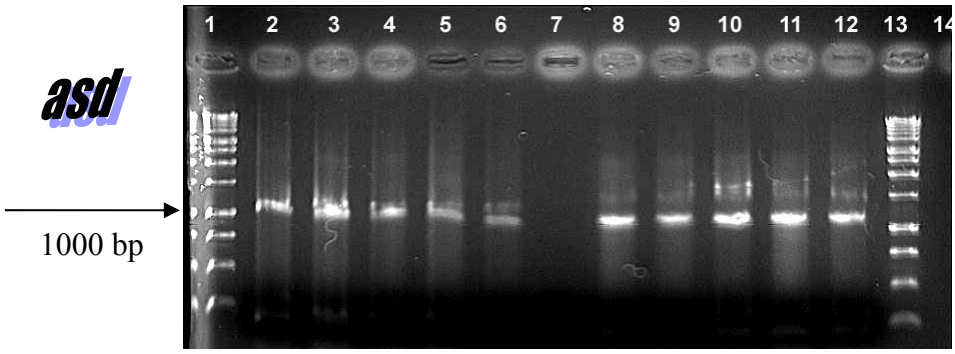

Figure 2-15. PCR product for *asd* gene in 9 clinical salmonella isolates (same isolates and orientation as Figure 2-14). Refer to Appendix C for specific isolate information. Lanes: 1) 1-kb ladder DNA marker (Promega), 2) χ3337 *spv* negative control, 3) χ3306 *spv* positive control, 4) Case 86, 5) Case 87, 6) Case 88, 7) Case 117, 8) Case 100, 9) Case 101, 10) Case 102, 11) Case 103, 12) Case 104, 13) 1-kb ladder DNA marker (Promega), 14) blank.

Figure 2-15 shows the PCR products of the salmonella *asd* gene for the same isolates (and same orientation in the gel) as Figure 2-14. Note that there is no product visible for the isolate in lane #7 of either Figure 2-14 or 2-15. This isolate was positively identified as *S.* Newport previously; however, the sample taken for template DNA in the PCR mixture on this day was taken from a HE plate—where *Salmonella* spp. are identified based on their ability to produce hydrogen sulfide. Normally these samples were taken from isolates growing on LB plates. Apparently the hydrogen sulfide or some other compound in the culture medium interfered with the PCR reaction, which would have caused a false-negative result to be generated had this control not been run simultaneously. An isolate was only evaluated for *spv* genes pending positive determination of the *asd* gene, which essentially validated that the isolate was a *Salmonella* spp.

Figure 2-16 demonstrates five clinical salmonella isolates that were positive for the *spvA* gene. Serovars represented by these five isolates include *S*. Typhimurium and 4,5,12:i-monophasic.

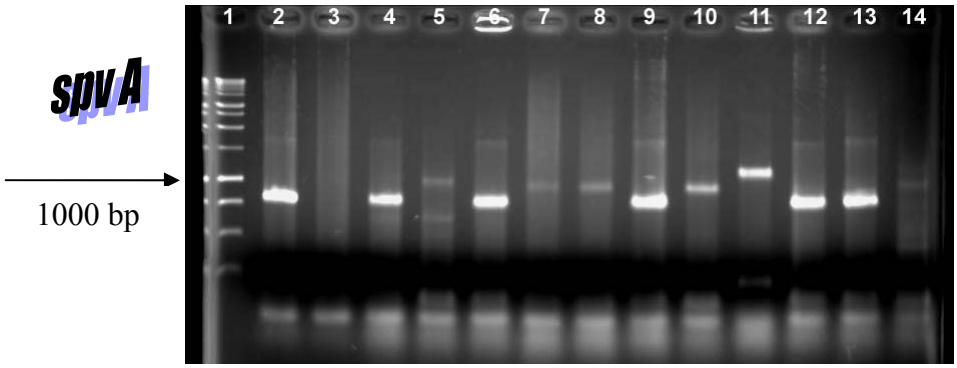

Figure 2-16. PCR product results for *spvA* genes in 11 clinical salmonella isolates, with positive and negative controls. Refer to Appendix C for specific isolate information. Lanes: 1) 1-kb ladder DNA marker (Promega), 2) χ3306 *spv* positive control, 3) χ3337 *spv* negative control, 4) lost isolate, 5) Case 8, 6) Case 7, 7) Case 12, 8) Case 13, 9) Case 10, 10) Case 9, 11) Case 5, 12) Case 3, 13) Case 11, 14) Case 4.

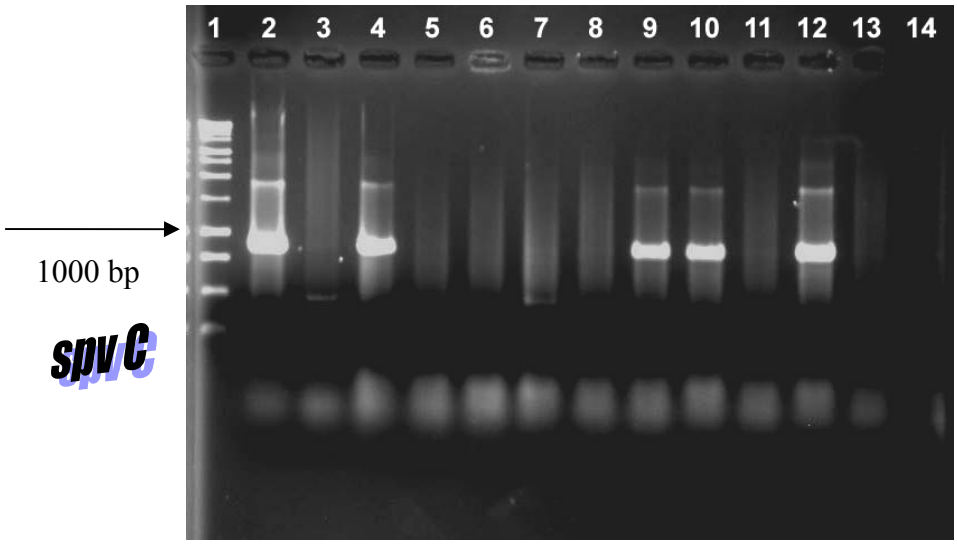

Figure 2-17. PCR product results for *spvC* genes in 11 clinical salmonella isolates, with positive and negative controls. Refer to Appendix C for specific isolate information. Lanes: 1) 1-kb ladder DNA marker (Promega), 2) χ3306 *spv* positive control, 3) χ3337 *spv* negative control, 4) Case 21, 5) Case 19, 6) Case 16, 7) Case 22, 8) Case 27, 9) Case 26, 10) Case 25, 11) Case 24, 12) Case 23, 13) Case 15, 14) Case 14.

Figure 2-17 demonstrates four clinical salmonella isolates that were positive for the *spvC* gene. Serovars represented by these four isolates include *S.* Typhimurium var. Copenhagen, *S.* Typhimurium, and 4,5,12:i-monophasic.

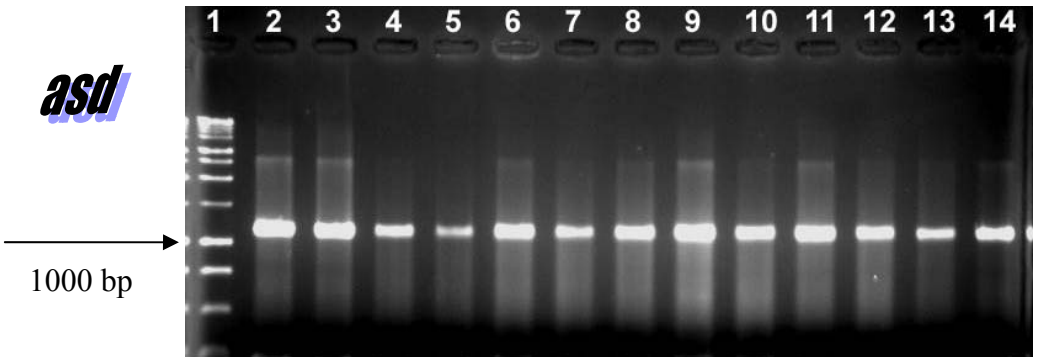

Figure 2-18. PCR product results for the *asd* gene in 11 clinical salmonella isolates (same isolates and orientation as Figure 2-17). Refer to Appendix C for specific isolate information. Lanes: 1) 1-kb ladder DNA marker (Promega), 2) χ3306 *spv* positive control, 3) χ3337 *spv* negative control, 4) Case 21, 5) Case 19, 6) Case 16, 7) Case 22, 8) Case 27, 9) Case 26, 10) Case 25, 11) Case 24, 12) Case 23, 13) Case 15, 14) Case 14.

Figure 2-18 shows the PCR products of the salmonella *asd* gene for the same isolates (and same orientation in the gel) as Figure 2-17. This is the typical appearance of *Salmonella* spp. probed with the *asd* primer set. All of these isolates could subsequently be examined for the *spv* genes since they were positively determined to be salmonellae.

Outcome by Presence of the Virulence Plasmid or *spv* Genes

Short-term outcome was examined with respect to the presence or absence of the virulence plasmid or *spv* genes. Results are detailed in Table 2-15.

Table 2-15. Summary outcome as determined by presence of the virulence plasmid and *spv* genes in 98 equine salmonella cases

	***spv*$^+$**	***spv*$^-$**	**TOTAL**
Lived	2	47	**49**
Died	9	28	**37**
Unknown Outcome	2	10	**12**
TOTAL	**13**	**85**	

There was a significant correlation between the presence of *spv* genes and mortality in this study population (p=0.001). Table 2-16 and Figures 2-19 and 2-20 depict the differences in short-term outcome between cases with respect to the virulence plasmid and *spv* genes. Nine out of 11 cases (81.8%) with *spv*-positive salmonella strains had a

fatal outcome as opposed to 28/75 (37.3%) of the cases with *spv*-deficient strains. The *spv* genes were restricted to group B salmonellae including serovars Typhimurium, Typhimurium var. Copenhagen, and 4,5,12:i-monophasic. Horses infected with *spv* gene-positive salmonella serovars were 12.3 times more likely to die than if they were infected with a *spv* negative strain. Also, if the organism was detected outside the gastrointestinal tract it was significantly more likely to be *spv* positive.

Table 2-16. Effect of *spv* gene presence on mortality in 86 cases of equine salmonellosis where outcome was known*

Exposure	Died	Survived	% Dead	Odds Ratio	Lower 95%CI	Upper 95%CI
spv Positive	9	2	81.8	12.30	2.59	58.41
spv Negative	28	47	37.3	1.00	0.52	1.91

* Pearson Chi-square value of 14.070 with 1 degree of freedom, p=0.001.

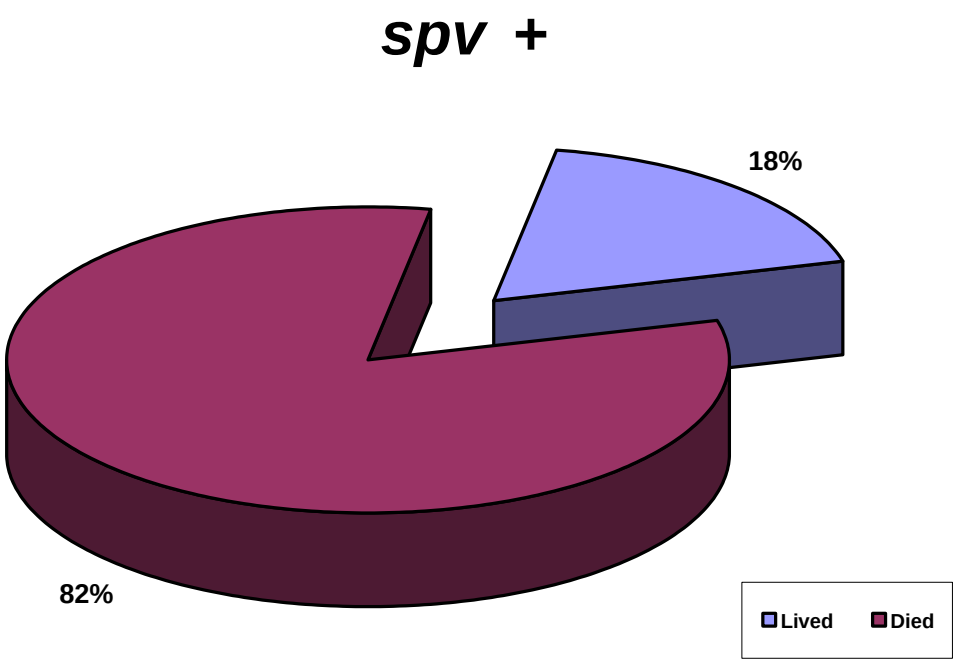

Figure 2-19. Outcome in equine salmonella cases, as influenced by presence of the *spv* gene locus.

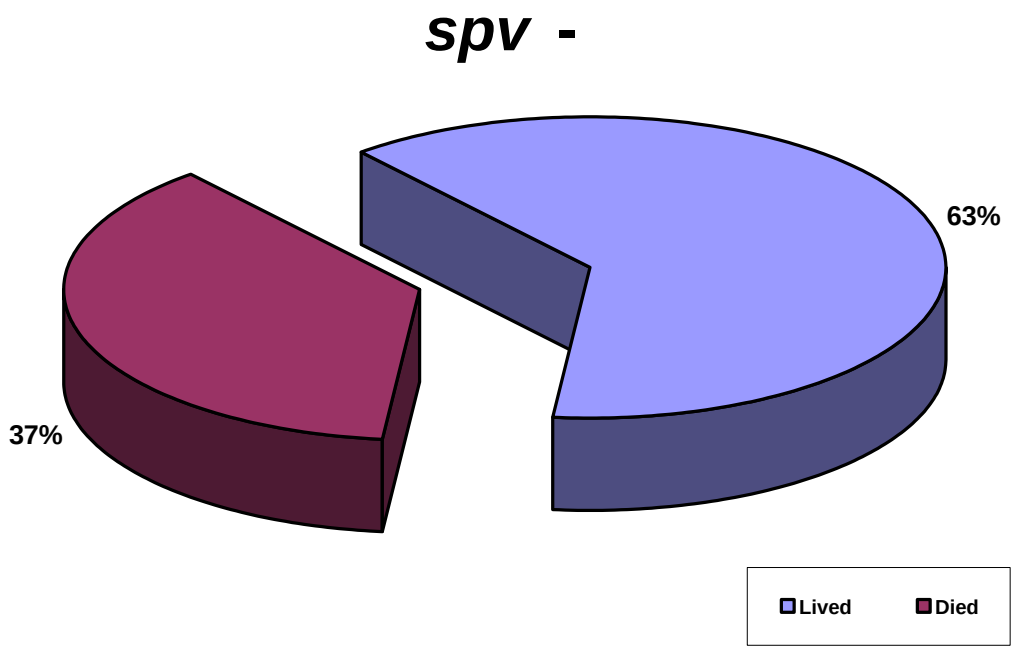

Figure 2-20. Outcome in equine salmonella cases, as influenced by absence of the *spv* gene locus.

Effect of Clinical and Laboratory Parameters on Outcome

A large number of independent clinical and laboratory variables were investigated with respect to predicting outcome in horses infected with *Salmonella*. Individual variables with a p-value less than 0.2 were included in the original model. There was no significant difference between survivors and non-survivors with respect to total serum protein (TSP) at admission (p=0.197) or TSP at death or discharge (p=0.198), however both factors significantly impacted outcome when investigated using forward stepwise logistic regression. The median TSP at admission and discharge for the survivors was 6.25 (mean = 6.45, with 6.1 to 6.6 95%CI) and 6.15 (mean = 6.1, with 5.9 to 6.4 95%CI) respectively. The median TSP at admission and death for the non-survivors was 6.8 (mean = 6.7, with 6.4 to 7.0 95%CI) and 5.5 (mean = 5.7, with 5.3 to 6.2 95%CI),

respectively. The total white blood cell and neutrophil counts at presentation did not predict outcome.

Regardless of clinical presentation or syndrome, the presence or absence of diarrhea was recorded where available for each case. True to the predominantly enteric nature of this disease, 74 out of 85 (87.1%) cases exhibited diarrhea at some point during hospitalization. Eleven cases (12.9%) did not develop diarrhea at any point during hospitalization, and the information could not be determined for 21 cases. The presence of absence of diarrhea did not predict survival.

Forward stepwise logistic regression analysis indicated that four categorical predictor variables had a significant impact on outcome: *spv* gene status, TSP at admission, TSP at death or discharge, and days of hospitalization were all related to outcome. The average number of days spent hospitalized was 10.2, with a minimum of one day (6 cases) and a high of 48 days. Of those cases that had a 3-day or less hospitalization period, there was a 91.66% mortality rate—these cases likely were admitted with severe disease, and were euthanatized due to expense, prognosis or complications, with the *Salmonella* not being confirmed until after death. The significant variables with test statistics are included in Table 2-17.

Table 2-17. Logistic regression model with variables predictive of outcome

Variable	B	SE	Wald Statistic	df	Significance	Exp (B)
***spv* genes**	-2.710	1.077	6.332	1	0.012	0.067
TSP at admission	1.050	0.398	6.967	1	0.008	2.859
TSP at death or discharge	-1.099	0.356	9.516	1	0.002	0.333
Days of hospitalization	-0.169	0.061	7.633	1	0.006	0.845

Relationship Between Proportion of Positive Fecal Salmonella Cultures and Outcome

A Kruskal-Wallis test was used to investigate the percentage of submitted samples that were positive with respect to outcome. There was a significant difference (p=0.042) between those that lived and those that died. Horses that survived had a median of 60% of their fecal cultures that were positive (mean = 62.71%, with 53.8% to 71.6% 95%CI), as compared to horses that died spontaneously or that were euthanatized, where the median percentage of positive cultures was 100% (mean = 77%, with 65.8% to 88.2% 95%CI).

Antibiotic Resistance Profiles

Antibiograms were obtained for 101 isolates. Complete MIC and resistance data for all isolates can be found in Appendix E. A summary of the antibiotic susceptibilities of 101 cases is displayed in Table 2-18 and the complete antibiogram of Case 78, demonstrating the only isolate with reduced sensitivity to the fluoroquinolone enrofloxacin, is shown in Table 2-19.

Table 2-18. Antibiotic susceptibilities for 101 equine salmonella isolates. The reported % susceptible, % intermediate, and % resistant, are only for those isolates with data for that antibiotic.

Antibiotic	% Susceptible	% Intermediate	% Resistant
Clindamycin	1.1	0	98.5
Erythromycin	1.15	0	98.5
Penicillin	0	0	100.0
Oxacillin	1.1	0	98.9
Rifampin	1.1	0	98.9
Doxycycline	58.9	0	41.0
Tetracycline	61.0	0	39.0
Trimethoprim-Sulfamethoxazole	61.4	0	38.6
Amoxicillin-Clavulanic Acid	67.4	0	32.6
Ampicillin	68.3	0	31.7
Ceftiofur	69.0	0	31.0
Cefazolin	70.0	2.0	28.0

Table 2-18. Continued

Antibiotic	% Susceptible	% Intermediate	% Resistant
Ceftazidime	70.0	10.0	20.0
Gentamicin	75.2	9.9	14.9
Chloramphenicol	85.0	1.0	14.0
Amikacin	92.9	0	7.1
Enrofloxacin	99.0	1.0	0
Imipenem	100.0	0	0
Nitrofurantoin	100.0	0	0

Table 2-19. Antibiotic susceptibility report for Case 78, with intermediate resistance to enrofloxacin

Drug	MIC	RS	Drug	MIC	RS	Drug	MIC	RS
Amikacin	<=2	S	Amox/Clav	>16	R	Ampicillin	>16	R
Cefazolin	>16	R	Ceftazidime	32	R	Ceftiofur	>4	R
Chloramp	32	R	Clindamy.	>2	R	Doxycyc.	>4	R
Enroflox.	1	I	Erythrom.	>4	R	Gentamicin	>8	R
Imipenem	<=1	S	Nitrofur.	<=32	S	Oxacillin	>4	R
Penicillin	>16	R	Rifampin	>4	R	Tetracyc.	>16	R
TMP-Sulfa	>4	R						

Antibiotic Resistance Transformation

Electroporation transformation was successful in transferring cefazolin resistance from the three salmonella isolates; but arcing due to the presence of buffer salts could have potentially damaged the plasmid DNA to the extent of generating false-negative results. Chemical transformations were performed with successful transference of cefazolin, ceftiofur and ampicillin resistance from all three isolates. Figures 2-21 through 2-24 show the gel electrophoresis results for these analyses. Cefazolin-resistant *E. coli* was shown to contain a new plasmid equivalent in size to the original cefazolin-resistant transforming salmonella isolate (Figure 2-21). The untransformed *E. coli* did not possess a plasmid, and is included for comparison.

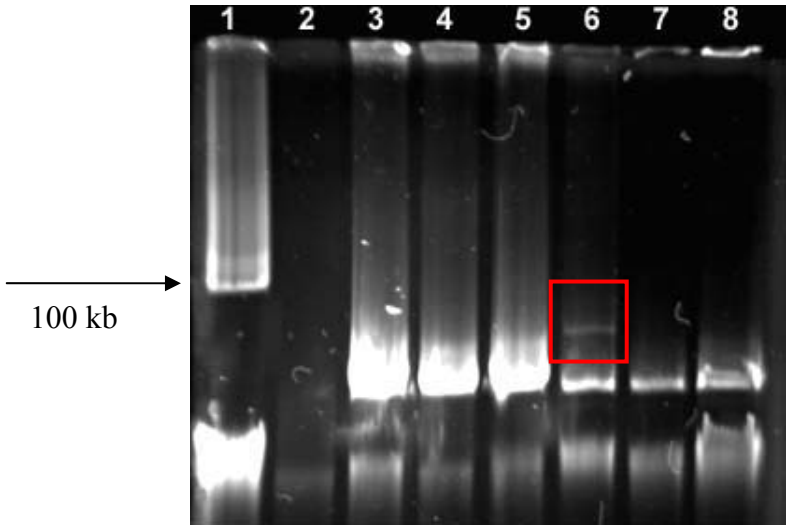

Figure 2-21. Plasmid profiles of 3 clinical salmonella isolates and *E. coli* transformed with plasmid DNA from those isolates. Refer to Appendix C for specific isolate information and Appendix E for antimicrobial susceptibilities. Lanes: 1) 100-kb plasmid of χ3306, 2) Untransformed *E. coli* DH5α, 3) *E. coli* DH5α transformed with Case 97, grown in CEF, 4) *E. coli* DH5α transformed with Case 92, grown in CEF, 5) *E. coli* DH5α transformed with Case 98, grown in CEF, 6) Transforming plasmid DNA from Case 97, 7) Transforming plasmid DNA from Case 92, 8) Transforming plasmid DNA from Case 98.

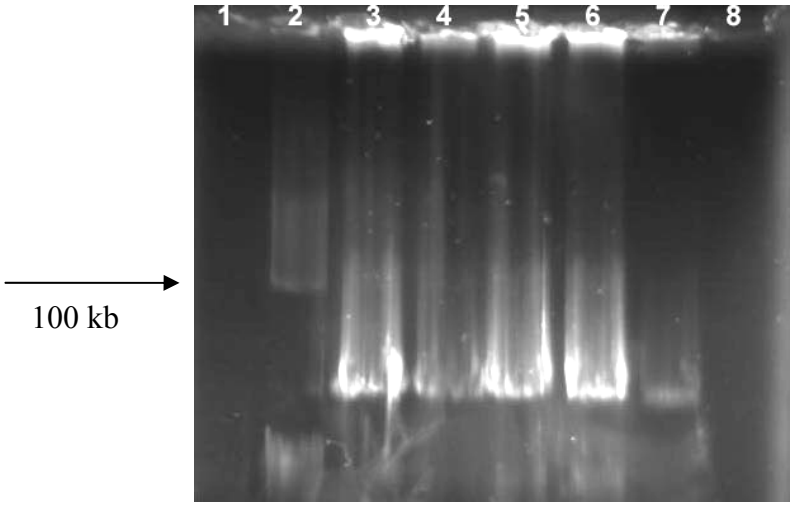

Figure 2-22. Plasmid profiles of 2 clinical salmonella isolates and *E. coli* transformed with plasmid DNA from those isolates. Refer to Appendix C for specific isolate information and Appendix E for antimicrobial susceptibilities. Lanes: 1) Untransformed *E. coli* DH5α, 2) 100-kb plasmid of χ3306, 3) *E. coli* DH5α transformed with Case 98, grown in AMP, 4) *E. coli* DH5α transformed with Case 98, grown in NAX, 5) *E. coli* DH5α transformed with Case 98, grown in CEF, 6) *E. coli* DH5α transformed with Case 92, grown in AMP, 7) *E. coli* DH5α transformed with Case 92, grown in CEF, 8) blank.

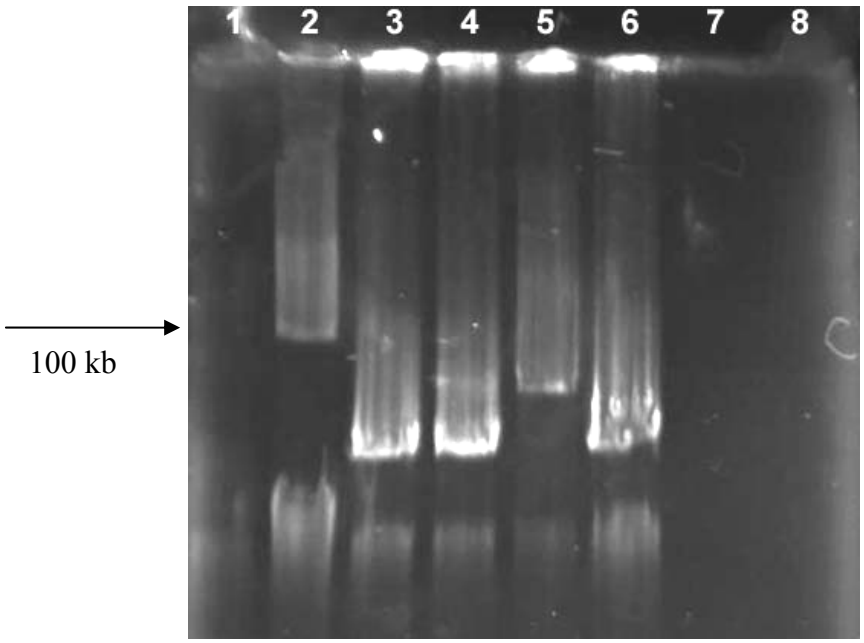

Figure 2-23. Plasmid profiles of 2 clinical salmonella isolates and *E. coli* transformed with plasmid DNA from those isolates. Refer to Appendix C for specific isolate information and Appendix E for antimicrobial susceptibilities. Lanes: 1) Untransformed *E. coli* DH5α, 2) 100-kb plasmid of χ3306, 3) *E. coli* DH5α transformed with Case 97, grown in AMP, 4) *E. coli* DH5α transformed with Case 92, grown in NAX, 5) *E. coli* DH5α transformed with Case 97, grown in NAX, 6) *E. coli* DH5α transformed with Case 97, grown in CEF, 7) blank, 8) blank.

In Figure 2-23, lane 5 shows an isolate (Case 97) that transferred resistance to NAX, CEF, and AMP. The plasmid transferring resistance to ceftiofur is larger than the other two transforming plasmids (which appear to be the same size). Looking at the plasmid profile of Case 97 in Figure 2-24, lane 5—there are three large plasmid bands visible (2 smaller than 100-kb and 1 larger). This isolate most likely is carrying the AMP and CEF resistance genes on the same plasmid and the NAX resistance gene on another larger plasmid. On closer examination of Figure 2-21—red box, the second larger plasmid is visible in Case 97 (lane 6), along with the other transforming plasmids of homogenous size.

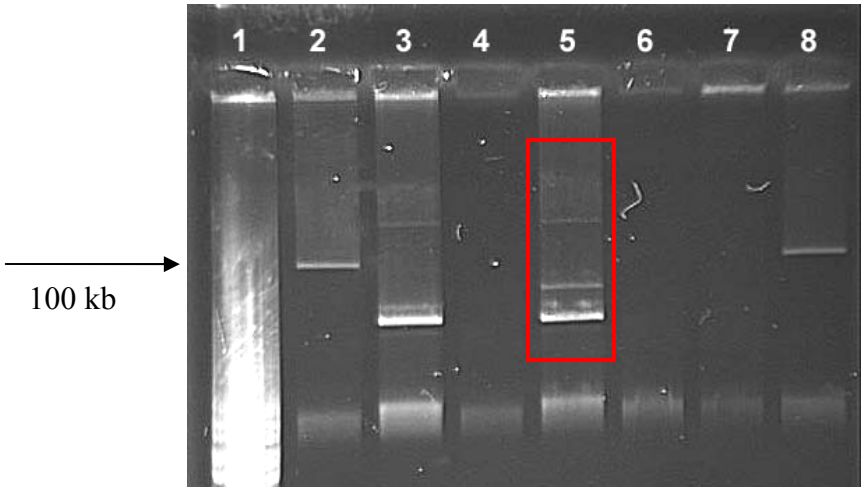

Figure 2-24. Plasmid profile of Case 97—lane 5. The red box delineates 3 large plasmid bands that are visible in the upper part of the lane. This isolate transferred ceftiofur, cefazolin, and ampicillin resistance via two different plasmids (the lower two).

These experiments demonstrate that cefazolin, ampicillin, and ceftiofur resistance in Cases 92, 97, and 98 were carried on plasmids which are smaller than the 100-kb virulence plasmid and do not contain the *spv* genes.

Site of Salmonella Isolation

The majority of isolates in this study were obtained from fecal samples (80.0%). Isolates from various segments of the gastrointestinal tract were examined separately and as part of the group of gastrointestinal isolates. If isolates from all enteric sites are considered together, the proportion of gastrointestinal isolates in the study rises to 93.3%. Isolate distribution by site of infection is summarized in Table 2-20.

Table 2-20. Clinical salmonella isolates from 105 equine cases by location of cultured specimen

Anatomic Site	Number of Cases	% of Total Cases
Feces	84	80.0
Small Intestine (necropsy or surgery)	7	6.7
Large Intestine (necropsy)	4	3.8
Synovial (joint) Fluid	2	1.9
Lung (necropsy)	1	1.0

Table 2-20. Continued

Anatomic Site	Number of Cases	% of Total Cases
Duodenum (necropsy)	1	1.0
Gastric Reflux	1	1.0
Abscess	1	1.0
Rectal Biopsy	1	1.0
Blood	1	1.0
Liver (necropsy)	1	1.0
Physis (necropsy)	1	1.0

The relationship between serovar and site of infection is summarized in Table 2-21. Figure 2-25 illustrates the systemic isolates compared to the gastrointestinal isolates by group.

Table 2-21. Systemic sites of salmonella infection in horses by serovar

Serovar	Number and % of Systemic Isolates
Hartford	1/1 100.0
Typhimurium	3/8 37.5
Muenchen	1/5 20.0
Typhimurium var. Copenhagen	1/7 14.3
Newport	0/13 0.0
Java	0/23 0.0
Javiana	0/7 0.0
Miami	0/7 0.0
Saintpaul	0/6 0.0
Anatum	0/4 0.0
4,5,12:i-monophasic	0/3 0.0
Newington	0/2 0.0
London	0/2 0.0
Mbandaka	0/2 0.0
Agona	0/1 0.0
Braenderup	0/1 0.0
Infantis	0/1 0.0
Meleagridis	0/1 0.0
Reading	0/1 0.0
Rubislaw	0/1 0.0
Tallahassee	0/1 0.0

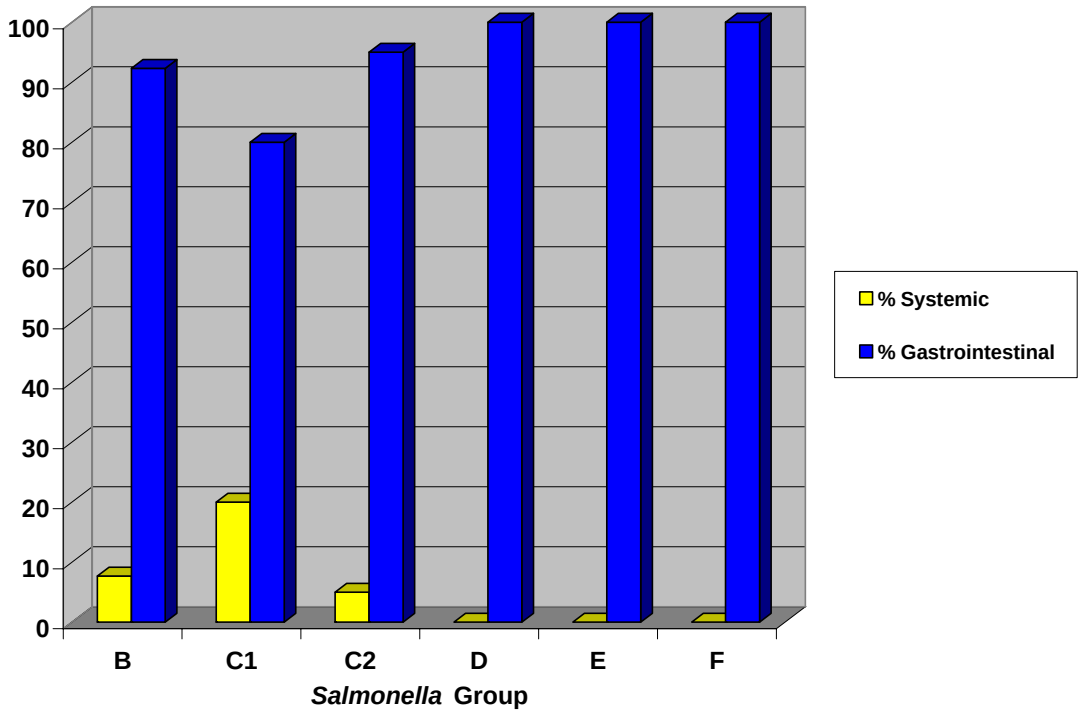

Figure 2-25. Systemic equine salmonella isolates compared to gastrointestinal isolates by group

Extra-intestinal isolates were 16.18 times more likely to carry the *spv* genes on a virulence plasmid than enteric isolates (p=0.001). All salmonellae that contained the *spv* genes also carried a large plasmid; no isolates were plasmid-negative and *spv*-positive. This was significant with a 95% confidence interval of 3.54 to 74.06. Data are summarized in Table 2-22.

Table 2-22. Relationship of the virulence plasmid and *spv* genes to isolate location in 98 cases of equine salmonellosis*

Site of Isolation	*spv* Positive	*spv* Negative	% Positive	Odds Ratio	Lower 95%CI	Upper 95%CI
Extra-intestinal	4	3	57.1%	12.15	2.34	63.10
Intestinal	9	82	9.9%	1.00	0.38	2.65

* Pearson Chi-square value of 18.994 with 1 degree of freedom, p=0.001.

Multi-Serovar Salmonella Infections

Six horses had more than one serovar of *Salmonella* isolated from them during hospitalization. All of the horses with multi-serovar salmonella infections developed or were admitted with diarrhea, all survived, and all of the isolates were obtained from fecal specimens. Table 2-23 details the groups, serovars, and relevant case information from these 6 horses.

Table 2-23. Details of multi-serovar salmonella infections in six horses 1999-2002

Case ID	Sex	Age	Specimen Origin	Salmonella Group	Serovar	Clinical Syndrome	Outcome	Diarrhea
80	M	7y	Feces	C2	muenchen	Diarrhea, Fever	Lived	YES
36			Feces	F	rubislaw			
70	M	6y	Feces	B	java	Post-Op Colic Diarrhea	Lived	YES
69			Feces	D	javiana			
52	F	3m	Feces	C2	newport	Diarrhea, Fever	Lived	YES
58			Feces	D	miami			
75	M	3m	Feces	B	java	Diarrhea	Lived	YES
71			Feces	D	miami			
73	F	6y	Feces	C1	hartford	Diarrhea, Fever	Lived	YES
39			Feces	C2	newport			
81	M	3m	Feces	B	Multiple serovars (NVSL sample)	Colic - Diarrhea, Chronic Diarrhea	Lived	YES
37			Feces	C2	muenchen			

Discussion

Risk factors for the development of salmonella infection in horses have been well described and were not investigated in the present study. The original aim of the proposed study was to contrast *Salmonella* spp. shed from diseased animals in a hospital setting with those recovered from a population of asymptomatic animals at pasture. This comparison was to focus on isolate serovar, grouping, plasmid, and *spv* gene status. Unfortunately, despite extensive and repeated culturing of animals at pasture we were unable to isolate *Salmonella* from any asymptomatic animal. This finding was a surprise, even in the face of a low prevalence (0.8%) reported in the recent NAHMS survey of North American horses.[95] With prevalence estimations ranging between 0% to 70% of the horse population, depending on the risk group being sampled and the type of diagnostic test used, it was expected to find at least one horse asymptomatically shedding *Salmonella* in their feces. The sampling was done over several seasons to ensure that the influence of temperature, weather patterns and time of year was minimized, and several samples were taken from each animal over a period of time to maximize the possibility of identifying periodic shedding episodes. There are several explanations for this negative result: too few samples examined per horse, culture techniques too insensitive to identify the low levels of bacteria shed by healthy horses, or more likely, that the true prevalence was so low that insufficient numbers of animals were sampled. The sample collection procedure and culture techniques were validated with samples from hospital patients known to be shedding *Salmonella*. Enrichment (with sodium selenite ± cystine, or tetrathionate broth) and culture is currently the gold standard for diagnosing *Salmonella* from fecal samples in horses, nevertheless the technique is not 100% sensitive.[50]

The study focus shifted towards a closer examination of the hospitalized population, including descriptive data, risks factors within this population associated with outcome, including organism group, serovar and *spv* gene status. With respect to the descriptive data we were restricted by an inability to obtain accurate hospital population demographics for the period in question. The breed distribution likely reflected the regional and hospital population. No breed predilection has been reported for non-host adapted salmonella infection in similar populations of hospitalized horses.[44;136] The mean age of affected animals in the present study was low in comparison to published values, but likely reflects the referral horse population in North Central Florida. This teaching hospital has a large caseload of young horses and foals due to close proximity to breeding farms, and this factor likely contributed significantly to the low mean age. Twenty-eight cases (28.57%) were in horses less than 6 months old, consistent with the opportunistic nature of *Salmonella* in the very young, immunosuppressed, or geriatric animals.[107] Olsen et al. showed a very similar distribution regarding isolation rates by age in humans, with over 48% of 441,863 isolates coming from individuals less than 19 years of age.[137] The unbalanced distribution of the case population may also be a reflection of compounded risk factors associated with age (e.g., younger horses may undergo surgery more often than older ones, or younger horses are kept in larger groups and may have an increased exposure to pathogens relative to solitary individuals).

As expected and reported in the literature, the largest number of cases in the present study occurred during the warmer months of the year, 68% from April through September. The seasonal predominance of salmonellosis in horses is typically highest during the warmer summer months[95;99] and this seasonality was likely extended due to

the warm Florida climate. Thirty-year average minimum temperatures in Gainesville, Florida remained above 60.3˚F (15.7˚C) during the months of May through October (Table 2-10).The wide spectrum of *Salmonella* recovered in this population is consistent with previous studies in horses. There were significant associations between salmonella grouping and *spv* gene presence and mortality. Animals infected with group B *Salmonella* were nearly 16 times more likely to die than infected with the common Group D bacteria. It was not surprising that the highest percentage of non-survivors occurred in the group B organism *S*. Typhimurium and related serovar groups *S*. Typhimurium var. Copenhagen and 4,5,12:i-monophasic. *S*. Typhimurium is a serious pathogen worldwide, with higher mortality rates than many other serovars, even within the group B. *S*. Typhimurium and *S*. Typhimurium var. Copenhagen were shown to cause significantly higher fatality rates than all other serovars in two studies of hospitalized horses.[99;138] This effect could likely be attributed to the presence of virulence plasmids, other antimicrobial resistance factors, or undetermined virulence factors significant in horses. Plasmid-bearing, *spv* gene positive organisms were restricted to group B *Salmonella*. Eighty seven and a half percent of *S*. Typhimurium isolates were *spv* gene positive; 29% of *S*. Typhimurium var. Copenhagen isolates were *spv* gene positive; and all 3 isolates of 4,5,12:i-monophasic contained *spv* virulence genes. It is important to point out however that many group B *Salmonella* do not carry *spv* genes. This includes *S*. Java (none of 23 isolates), *S*. Saint Paul (0 of 6), *S*. Agona and *S*. Reading (0 of 1, respectively).

Extra-intestinal isolates were limited to groups B, C1, and C2. The serovars recovered from those isolates included *S*. Hartford, *S*. Typhimurium, *S*. Muenchen, and *S*. Typhimurium var. Copenhagen. *S*. Typhimurium was the only serovar with more than

one systemic isolate, and more than 37% of all *S.* Typhimurium isolates were from systemic sites. Systemic isolates had a significantly higher potential of carrying the *spv* genes. This finding may indicate a similar role for the salmonella virulence plasmid and these genes in horses, as demonstrated in calves,[26] humans,[22;139] and mice.[21] Montenegro et al. showed that virulence plasmids were detected in nearly 100% of extra-intestinal isolates from human blood as well as cattle or swine internal organs.[23]

In summary, *spv* gene-containing isolates in horses are likely restricted to certain group B salmonellae, are more likely to be recovered outside the intestinal tract, and are more commonly associated with a negative outcome than non-*spv* gene-containing isolates. The fact that all *spv* positive isolates were Group B salmonellae is also in agreement with published reports. Eleven different serovars have been reported to carry virulence plasmids (including *S.* Typhimurium); however, not all isolates within those serovars will necessarily contain a virulence plasmid.[140] In the present study, one *S.* Typhimurium and six *S.* Typhimurium var. Copenhagen isolates did not possess the *spv* genes.

Verification that the *spv* genes were located on the plasmid (and not integrated into the chromosomal DNA) was not performed, but could be determined by transferring the gel electrophoresis products to solid membranes, and then DNA-DNA hybridization to the plasmid band (Southern blot). Chromosomal integration of the *spv* genes has only been reported in subspecies II, IIIa, IV, and VII which typically infect cold-blooded vertebrates.[141;142] These subspecies do not infect warm-blooded vertebrates—only subspecies I isolates have demonstrated mammalian pathogenicity.[134] It was also shown in a mouse-avirulent subspecies IV isolate that the chromosomally integrated *spv* genes

were not normally expressed and complementation with the entire virulence plasmid from *S.* Typhimurium did not cause the isolate to become mouse virulent.

The ability to recover through bacterial culture, *Salmonella* spp. from fecal samples, correlated with outcome. In general, animals with significant enteric disease and higher mortality were more likely to return a larger proportion of positive cultures than those with milder disease. This may be related to the number of organisms being shed and/or to the immune status of the animal, with immunocompromised individuals unable to significantly respond to the organism.

A recent retrospective study determined that low serum total protein concentrations were associated with failure to survive in horses admitted for acute diarrhea.[143] Using limited clinical and laboratory data we performed a stepwise logistic regression analysis in order to unmask factors that may be important in predicting outcome in horses with salmonella infection. We also concluded that total plasma protein was an important determinant of outcome, in addition to *spv* gene status, and duration of hospitalization. Unfortunately none of these factors, with the exception of total plasma protein at admission, could be used reliably to predict outcome in the clinical setting. The protein concentration at admission was higher in the non-surviving group, likely reflecting more severe hemoconcentration in those horses associated with acute fluid losses. Although this finding is of clinical interest it is unlikely by itself to influence the decision to pursue treatment. Overall, *spv* gene-containing isolates in horses are likely restricted to group B organisms, more likely to be recovered outside the intestinal tract, and more commonly associated with a negative outcome than *spv* gene-negative isolates.

Examination of *in vitro* salmonella sensitivity data is an important facet of clinical practice. Not only does sensitivity data guide therapy but also is important in terms of monitoring for drug resistance. The recognition of fluoroquinolone resistance in resident strains of *Salmonella* is particularly important. A recent report detailed an outbreak and general increase in the number of multidrug-resistant *S*. Newport being isolated from humans.[144] These isolates were resistant to amoxicillin/clavulanate, ampicillin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline, and the resistance can be attributed to the presence of plasmids carrying a bla_{cmy} gene, which produces AmpC-type enzymes that confer resistance and are termed Newport MDR-AmpC strains. One isolate (case 14) was from a horse previously treated with antibiotics (penicillin and trimethoprim-sulfamethoxazole) that developed diarrhea attributed to *S*. Newport. This isolate had a resistance pattern strikingly similar to the multidrug-resistant *S.* Newport described in the report (resistant to amoxicillin/clavulanate, ampicillin, cefazolin, ceftazidime, ceftiofur, chloramphenicol, clindamycin, doxycycline, erythromycin, oxacillin, penicillin, rifampin, tetracycline, and trimethoprim-sulfamethoxazole) and also carried a large plasmid that did not contain the *spv* genes. These strains of *Salmonella* are commonly associated with dairy farms, sick cows, and unpasteurized milk or cheese.[144]

Only four serovars of *Salmonella* accounted for the 22 multidrug-resistant isolates identified in this study (resistant to ≥8 drugs out of the 12 clinically relevant drugs tested). *S.* Java accounted for ten, *S.* Typhimurium var. Copenhagen accounted for five, *S.* Javiana accounted for two, and *S.* Newport accounted for one. Interestingly, *S.* Typhimurium var. Copenhagen was also isolated from four hospitalized cows during the

same time period, and similar to what was reported for *S.* Newport, dairy cattle could be reservoirs as well as modulators of resistance pressure in this serovar. It should be noted that *in vitro* sensitivity data does not directly correlate with *in vivo* susceptibility due to the normally intracellular location of this organism. This is particularly true for non-lipid soluble antibiotics such as gentamicin. Based on this population of organisms and their susceptibility data, clindamycin, erythromycin, penicillin, oxacillin, and rifampin cannot be recommended for therapeutic treatment of salmonella infections, due to more than 95% of all isolates being resistant to each of those drugs. This is expected based on the mode of action and gram-positive bacterial spectrum of these drugs. Drugs typically used for peri-operative prophylaxis such as the first generation cephalosporin cefazolin or the aminoglycoside gentamicin, had approximately 28% and 15% resistance respectively. Amikacin, enrofloxacin, imipenem, and nitrofurantoin each had greater than 92% of all isolates susceptible. A popular antibiotic selection for treatment of salmonella infections in adult horses is the fluoroquinolone enrofloxacin. Enrofloxacin has an excellent gram-negative spectrum, is accumulated within macrophages, and is effective against intracellular organisms such as *Salmonella*. In this study, a single isolate demonstrated intermediate resistance to this drug (Case 78); all others were susceptible. Case 78 was from an 18y old Welsh Pony that presented for a gastric impaction. Post-operatively, this horse developed diarrhea associated with a group B—*S.* Java, and was later euthanatized. This patient was treated with penicillin, gentamicin, and metronidazole during hospitalization and also was treated with enrofloxacin for the *Salmonella*. Surveillance for fluoroquinolone resistance in *Salmonella* (especially veterinary isolates) is extremely important, as these drugs are reserved for life-threatening infections in humans.[145-147]

Antibiotic resistances shown to be carried on plasmids include beta-lactamases and extended-spectrum beta-lactamases,[148] ampicillin,[149] tetracycline,[150] quinolones,[151] trimethoprim and sulfonamides.[149;152] The 3 cases demonstrating successful transference of antibiotic resistances were all the same serovar (*S.* Java), a prevalent serovar in the hospital during that time period. Prescott reported that multiple-antibiotic resistance is a problem only in *S.* Typhimurium and not in other salmonella serovars.[153] This was found not to be the case, as most of the multiple-antibiotic resistant serovars in this study were not *S.* Typhimurium, but *S.* Java. Preliminary data generated by this study supports the contention that the majority of large plasmids associated with clinical isolates of *Salmonella* from horses are likely antimicrobial resistance plasmids or R plasmids.

CHAPTER 3
EXPERIMENTS

Background

The Horse: Classic Large Intestine Fermenter

The horse is exquisitely dependent on the protozoal and microbial population within its cecum and large colons to ferment otherwise indigestible cellulose foodstuffs, similar to the forestomach ecosystem in ruminants.[154] The cecum in the horse and pony is analogous to the rumen, and SCFA production and metabolism in the cecum alone have been shown to supply approximately 30% of a horse's daily digestible energy intake.[155] This biological fermentation vat works best under the ideal conditions of constant influx of substrate and consumption or efflux of by-products; i.e., the horse is best suited to eat on a continuous basis, as opposed to the meal feeding activities of omnivores and carnivores.[156] Domestication of the horse and modernization of the horse industry have significantly changed management strategies, specifically diet composition and practices associated with feeding those diets. An eventual migration toward confinement, structured exercise, and concentrate meal feeding has exaggerated problems uncommonly seen in wild horse populations, such as colic, gastric ulcer disease, and infectious diarrhea.[157] Significant differences in dietary composition would theoretically have great impact on the amount of SCFA in the cecum and colon, but surprisingly, this is not the case. It has been shown that horses fed concentrated grain and carbohydrate-rich diets do not produce excessive amounts of SCFA as compared to horses fed control diets,[155;158] but they can have significantly different SCFA ratios than horses consuming

exclusively forage based diets or grazing.[159] These ponies developed lower acetate and higher propionate molar proportions on increased carbohydrate diets. The results in equine research agree with human studies, where no appreciable effects of dietary carbohydrate content on fecal SCFA concentrations were identified, but differences in the relative proportions of SCFA were reported.[160] Abrupt dietary decreases in the proportions of resistant starches to fermentable carbohydrates, however, can alter the microbiota and local climate of the cecum, such as decreasing the pH and total protozoal counts and increasing the numbers of lactate-producing anaerobes.[161;162] The effect of dietary manipulation of the intestinal microenvironment, particularly with respect to growth, attachment, and invasion of pathogens, is worthy of further investigation.

Equine Cecal Anaerobic Flora and SCFAs in the Normal Animal

A summary of the reported values for total culturable anaerobes in the equine cecum are detailed in Table 3-1 and a summation of several reports in the literature measuring equine cecal SCFA concentrations in normal animals is shown in Table 3-2.

Table 3-1. Summary of literature reports quantifying equine cecal anaerobic bacteria

Author	**Total Bacteria and Units of Measurement**	**Fistulated or Whole Animal**
Maczulak et al.[163]	DMC = 2.37 – 4.72 x 10^9 per ml Culture = 1.86 – 3.65 x 10^8 per ml	Fistulated
Kern et al.[164]	DMC = 458 – 702 x 10^7 per gram Culture = 35 - 181 x 10^7 per gram	Whole
McCreery et al.[165]	DMC = 10^{10} - 10^{11} per gram	
Mackie and Wilkins[166]	Culture = 25.85 x 10^8 per gram	Whole
Kern et al.[154]	DMC = 642 x 10^7 per gram Culture = 492 x 10^7 per gram	Whole
Julliand et al.[167]	Culture = 4.2 x 10^8 per ml	Fistulated
Goodson et al.[162]	Culture = 10^4 per gram	Fistulated
Medina et al.[168]	Culture = 2.4 x 10^8 per ml	Fistulated

Table 3-2. Literature reports quantifying normal equine cecal SCFA concentrations

	Acetate	Butyrate	Propionate	TOTAL
Glinsky[155]	73.7 % 51.00 mM	9.5 % 6.57 mM	16.7 % 11.56 mM	69.2 mM
Eldsden[41]	73.2 %	7.2 %	19.65 %	
Kern[164]	43.08 μM/ml	4.03 μM/ml	13.48 μM/ml	70.00 μM/ml (≈ mmol/L)
Kern[154]	74.7% 72.76 μM/g	5.6% 5.45 μM/g	18.4% 17.92 μM/g	97.4 μM/g
Mackie and Wilkins[166]	99.9 mM	3.8 mM	12.5 mM	118 mM
de Fombelle[169]	71.99 % 57.75 mmol/L	6.96 % 5.60 mmol/L	19.39 % 15.55 mmol/L	80.22 mmol/L
Horspool[170]	34.4 mmol/L 53.1 %	12.2 mmol/L 21.3 %	10.9 mmol/L 18.7 %	65.0 mmol/L
Medina et al.[168]	47.15 mM 70.2 %	3.86 mM 5.68 %	15.25 mM 22.15 %	67.3 mM
RANGE	**34.4 – 99.9 mM**	**3.8 – 12.2 mM**	**10.9 – 15.5 mM**	**65 – 118 mM**

Antimicrobial Effects on Normal Anaerobic Flora

Antimicrobial therapy at best is an artistic treatment modality, because its therapeutic effects extend to all susceptible bacteria living in or on the host, not just the pathogenic strains. Disruption of the normal commensal microenvironment can often be equally as detrimental to the host as the infection being treated. *In vitro* and *in vivo* methods have historically been used to examine the effects of antibiotic administration on the autochthonous flora of the gastrointestinal tract, an environment exquisitely dependent on the presence and activity of numerous species of commensal bacteria. Strictly anaerobic bacteria are responsible for many of the metabolic functions attributed to this ecosystem. Table 3-3 shows a current summary of multiple studies examining the effects of various antibiotics on fecal SCFA production (some in vitro studies are included) as well as effects on the levels of culturable aerobic and anaerobic bacteria in the feces. Overall, it is apparent that disruptions in the normal flora of the skin, mucous

membranes, gastrointestinal and urogenital tracts are common and direct effects of antibiotic administration, and that route, dose rate, mode of action, duration of therapy, and patient metabolic status are all important in mediating the particular sequence and severity of adverse effects attributed to a particular antimicrobial.

Table 3-3. Literature summary of antibiotic effects on fecal bacteria and short-chain fatty acids

Antibiotic	Species	Route of Administration	Duration of Treatment	Effect on Fecal Aerobe Culture*	Effect on Fecal Anaerobe Culture*	Effect on Fecal SCFA*
Amikacin	Equine Equine	Intravenous Oral	One dose One dose	---[171] ---[171]	---[171] --- to ↑[171]	---[171] ---[171]
Amoxicillin	Human	Oral	7 days	↓ to ---[172]	↓ to ---[172]	
Ampicillin	Human	Oral	5 days	↓↓↓[172]	↓↓↓[172]	
Bacitracin	Human Equine	Oral Oral	6 days 4 days	↓↓↓[86] (examined microflora associated functions – no cultures)	↓↓↓[86] (examined microflora associated functions – no cultures)	↓↓↓[82]
Ceftriaxone	Human	Intramuscularly	5 days	↓ to sl. ↑[85]	↓↓↓[85]	↓↓↓[85]
Clarithromycin	Human	Oral	7 days	↓↓↓ (E. coli)[88]	↓↓↓[88]	
Clinafloxacin	Human	Oral	7 days	↓↓↓[84]	↓↓↓[84]	
Clindamycin	Swine	*In vitro* Colon Simulation Technique (COSITEC)	5 days			↓↓↓ (*in vitro*)[92]
Co-triomoxazol (Trimethoprim-Sulfamethoxazole)	Human	Oral	6 days			↓ to ---[82]
Doxycycline	Human	Oral	6 days			↓ to ---[82]
Erythromycin	Human	Oral	6 days			↓↓[82]

Table 3-3. Continued

Antibiotic	Species	Route of Administration	Duration of Treatment	Effect on Fecal Aerobe Culture*	Effect on Fecal Anaerobe Culture*	Effect on Fecal SCFA*
Metronidazole	Swine	*In vitro* Colon Simulation Technique (COSITEC)	6 days			↓↓↓ (*in vitro*)[83]
Moxifloxacin	Human	Oral	7 days	↓↓↓[88]	↓ to ---[88]	
Nalidixic Acid	Human	Oral	6 days			↓ to ---[82]
Ofloxacin	Human	Oral	6 days			↓ to ---[82]
Penicillin	Human	Oral	>3 days			---[73]
Pivampicillin	Human	Oral	>3 days			---[73]
Streptomycin	Murine	Oral	7 days			↓↓↓[80]
Tetracycline	Human flora murine model	Oral	6 weeks	↓↓ to ↓[87]	---[87]	↓ to ---[87]
Trimethoprim-Sulfadiazine	Equine Equine	Intravenous Oral	5 days	↓↓ to ↓[49] ↓↓ to ↓[49]		
Vancomycin	Human Swine	Oral *In vitro* Colon Simulation Technique (COSITEC)	6 days 6 days			↓↓↓[82] ↓↓ (*in vitro*)[83]

*↓↓↓ = strong suppression, ↓↓ = moderate suppression, ↓ = minimal suppression, --- = no suppression, ↑ = increase

Antimicrobial Effects on SCFAs

The effects of orally administered antibiotics on gastrointestinal SCFAs are likely not a direct effect on the SCFAs themselves, but rather an effect on the organisms producing them. Several studies have shown that antibiotic administration has a significant effect on the distribution and concentration of SCFA in the feces of normal humans,[82;84;85] but not horses, although data are limited.[171] The effect of a single oral or intravenous dose of amikacin, an aminoglycoside with minimal predicted activity in an anaerobic environment such as the distal gastrointestinal tract, was examined in normal horses.[173] Another publication reported the effects of a single intravenous dose of oxytetracycline on cecal SCFAs in one pony, though the nature of the article was to validate methodology, and no interpretation was offered.[174] In one of the only comprehensive prospective studies involving *Equidae*, the investigator examined the effects of several antibiotics given by various routes, on cecal levels of SCFAs in horses, ponies, and donkeys.[170] This study found that antibiotic administration typically altered cecal and fecal SCFA levels by increasing lactic acid concentrations. Lactic acid is a SCFA but is considered to be a non-volatile fatty acid (NVFA) in contrast to acetate, propionate, and butyrate. A summary of that work is detailed in Table 3-4. The primary shortcoming of this research is that it only examined the effects of a single dose of antibiotic on the variables of interest. The effects of repeated dosing regimens are in need of investigation. Based on the paucity and impracticality of studies performed in the horse, as well as the contradictory nature of results in comparison to humans, the effects of antibiotic administration on equine gastrointestinal flora and SCFA profiles are yet to be examined.

Table 3-4. Summary of single dose antibiotic effects on the equine cecal microenvironment[170]

Drug	Route	Effect on Microflora*	Effect on pH*	Effect on SCFA*
Penicillin G	IV	---	---	↑↑ (lactic)
Penicillin G	Oral	↑↑ coliforms, streptococci, *Clostridium* spp.	↑↑	↑↑ (lactic), ↓↓ (butyric, propionic)
Ampicillin	IV	↑ to --- coliforms	---	↑↑ (lactic)
Ampicillin	Oral	↑↑ coliforms, streptococci, lactobacilli, *Clostridium* spp.	---	↑↑ (lactic), ↓↓ (propionic)
Amikacin	IV	---	---	---
Amikacin	Oral	---	↓↓	↑ (lactic)
Oxytetracycline	IV	---	---	↑↑ (lactic)
Oxytetracycline	Oral	↑↑ coliforms, streptococci, lactobacilli, *Clostridium* spp.	---	↑↑ (lactic)

*↓↓↓ = strong suppression / decrease, ↓↓ = moderate suppression / decrease, ↓ = minimal suppression / decrease, --- = no suppression / decrease, ↑ = increase

Current Theory on the Pathogenesis of Antibiotic-Associated Diarrhea (AAD)

Over the last 15 years, significant progress has been made in determining the risk factors, pathogenic mechanisms, therapies, and predictive outcomes regarding AAD. Focus has been primarily on the action or interaction of the antibiotic with the gastrointestinal flora and the repercussions of disturbing that ecosystem. Figure 3-1 shows a current summary assumption of the pathogenesis and progression of AAD. It is interesting to note that many of the processes involved in this pathogenesis are self-perpetuating, with the generation of cascading and cyclic effects on the entire host organism.

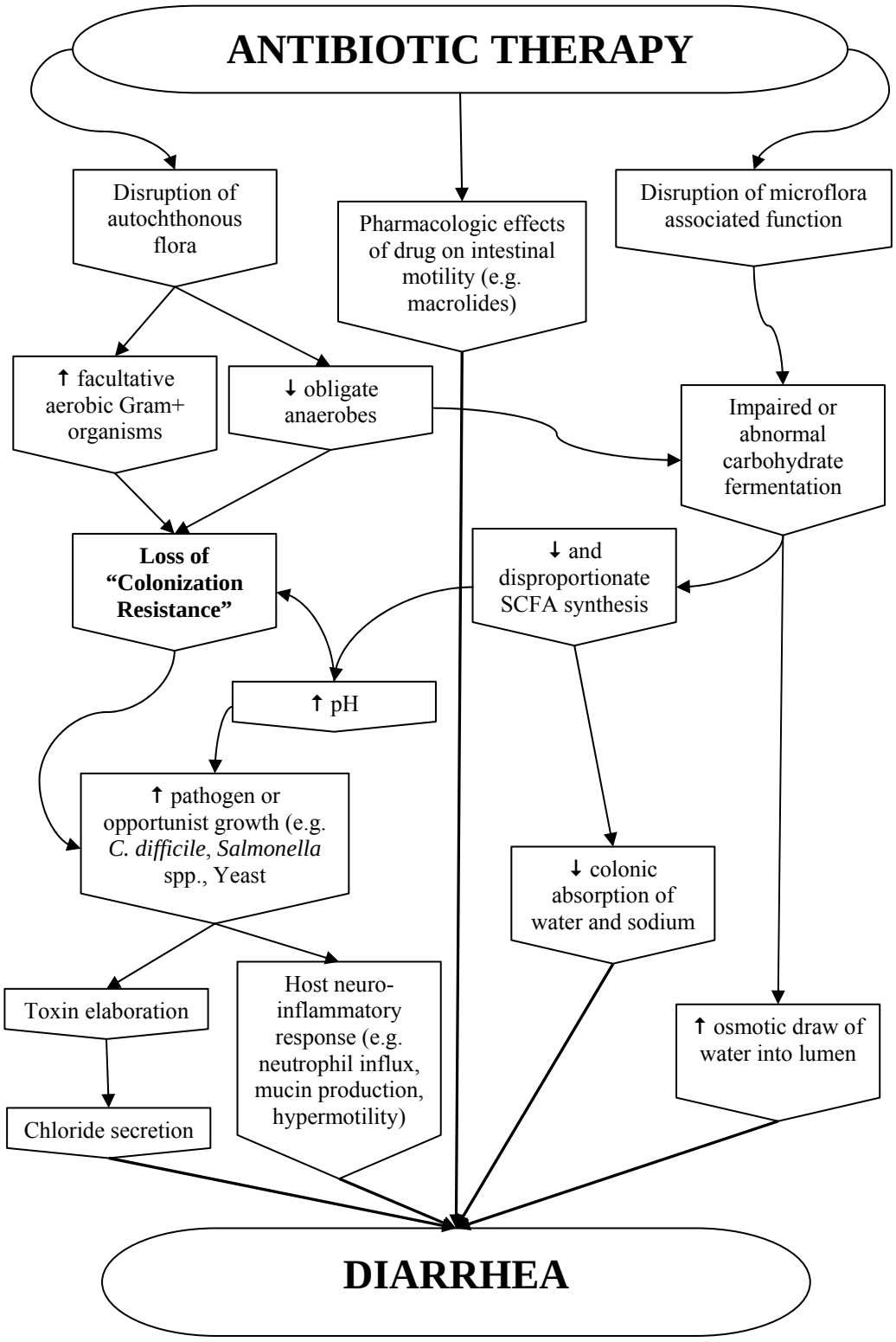

Figure 3-1. Pathogenesis of antibiotic-associated diarrhea. Adapted from Bergogne-Bérézin, 2000.[79]

Effects of SCFA on Anaerobic Growth of Bacteria

It has been thought that only the undissociated form of an organic acid is responsible for initiating an antimicrobial effect, by virtue of its ability to cross lipid membranes. Recent studies however, have shown that using the sodium salts of the acids have also demonstrated antimicrobial activity, and it is now known that both dissociated and undissociated lipophilic acids and bases can cross cell membranes according to concentration gradients, though the undissociated form is still the preferred route.[175] The sodium salts of SCFAs are nearly completely dissociated in solution. This dissociation behavior allows for an easily predictable amount of SCFA available to cross into the cytoplasm regardless of differences in local pH. Physiologically, as pH decreases, the amount of undissociated acid in a solution increases, and it is this undissociated (non-ionized) form ($HA \leftrightarrow H^+ + A^-$) of weak acids that is thought best able to penetrate the cell wall.[176] Once inside in the more alkaline cytoplasm of a cell, the acid again dissociates and there is a gradual accumulation of protons (H^+) and anions (A^-) within the cell, above what can be re-associated to intracellular cations such as potassium (K^+) and shuttled back out across the membrane.[175]

Acid Tolerance Response of *Salmonella* and Other Enterobacteriaceae

"What does not kill you makes you stronger" describes the ability of *Salmonella* to augment their resistance to organic acids or acidic pH after exposure to low pH alone[177] or in combination with individual SCFA.[178] This is termed the acid tolerance response (ATR) of *Salmonella*, which is evident in two distinct circumstances, while the cells are growing exponentially or while they are at stationary phase. It was also shown that this adapted acid resistance was further enhanced by increasing the length of exposure time to the SCFA, anaerobiasis, and acidic pH in conjunction with SCFA exposure.[178] Increased

protein synthesis was shown to be the basic mechanism responsible for the ATR, and over 50 distinct proteins have been termed acid shock proteins based on their synthesis response to low pH shock.[179] This ATR could have implications for the survival of *Salmonella* in the stomach, intestinal lumen, treated foodstuffs, silage, or in the phagosome of the macrophage. It was also shown that induction of the ATR in *Salmonella* indirectly confers protection to several other unrelated stressors, such as high osmolarity (2.5M NaCl) and reactive oxygen species (20mM H_2O_2).[180]

It has been demonstrated that concentrate feeding in cattle decreased large intestine pH and increased the relative concentrations of SCFAs, which induced an acid-resistant phenotype in the native *E. coli* (rather than selection for an acid-resistant sub-population).[181] This phenomenon could easily apply to the intestinal microflora and/or *Salmonella* in the case of the domestic horse.

Route of infection appears to be an important determinant of pathogen survival and ability to invade through the gastrointestinal barrier in salmonella infections, and may be associated with exposure to low pH. When *S*. Dublin is given orally[26;182] to calves, or S. Typhimurium is given orally to mice[89] or horses,[94;96] typical courses of disease result. Nicpon et al. conducted experiments utilizing chronically fistulated horses, where they were given large (100 ml of 10^8 cells per ml) challenge doses of virulent *S.* Typhimurium or *S*. Enteriditis orally or directly into the cecum.[183] Only the horses given *Salmonella* via the oral route developed any clinical signs such as fever, diarrhea, and colic. The horses given *Salmonella* via cecal fistula (two doses per horse) did not develop any signs of disease. This effect could be attributed to the ability of the host to overwhelm and

dispatch the minimally prepared and defended organism, or could be due to completely unrelated virulence factors.

SCFA Effects on Salmonella Growth and Invasion

"SCFA toxicity" is a term used to describe the bacteriostatic and bacteriocidal effects of SCFA on bacteria. This mechanism has historically been explained by an uncoupling theory, where an abnormally low luminal pH allows undissociated acid to pass through the cell membrane, become dissociated in the more alkaline interior of the cell, and abolish the proton-motive force across the cell membrane, thereby acidifying the cell and halting intracellular metabolism and protein synthesis. This theory does not adequately explain why many pathogenic and commensal cells are able to survive, continue to function or even flourish in the slightly acidic and SCFA rich environment of the mammalian large intestine. A newer theory has been proposed that can better explain the fermentation acid-resistant phenotype that some bacteria innately possess or develop with exposure to SCFA. This theory also explains the phenomenon of decreased epithelial cell association and decreased growth rates of *Salmonella* in low pH or SCFA containing culture media. pH Gradient-Mediated Anion Accumulation was defined in 1998 and suggests that SCFA resistant microbes have a reduced pH gradient across their cytoplasmic membrane (ΔpH) and higher intracellular concentrations of potassium ions to combine with the accumulating acidic anions.[175] Some more notable gram-negative bacteria such as *E. coli* O157:H7 have also refined their ability to decrease ΔpH, and are notorious foodborne pathogens.[175]

Acetate and formate tend to promote the invasive phenotype in different salmonella serovars, whereas butyrate and propionate tend to inhibit invasion.[184;185] Acetate is able

to cross bacterial cell wall membranes in the undissociated (non-ionized) form, whereas butyrate and propionate cannot.[186] It has therefore been suggested that acetate can initiate different cellular signals compared to butyrate or propionate.[185]

In vitro growth of *Salmonella* supplemented with biologically relevant SCFAs in physiologic and aphysiologic concentrations has been studied by several investigators. Unfortunately, most of this work has been done under aerobic conditions, and minimal data are available examining the effects of SCFAs under anaerobic growth conditions. The distal intestinal environment is completely anaerobic, and bacteria undergoing anaerobic respiration may respond very differently than in the presence of oxygen. pH is a critical factor in determining whether or not a SCFA at a particular concentration has an effect on salmonella growth. Durant et al. showed that under aerobic conditions at pH 6, growth rates of *S.* Typhimurium were decreased in acetate, butyrate, and propionate containing media (25, 50 and 100mM), while no differences were observed when the pH was raised to 7.[185]

SCFAs are weak acids with pKa's averaging 4.8 and according to the local pH will be present in either ionized or non-ionized forms, which should determine their absorption kinetics across biological membranes. Recently, the pH of the intestinal lumen has been shown not to influence the absorption of SCFAs in the guinea pig and human colon, nor in guinea pig and rat cecum models. This segregation of what was thought to be a dependent interaction is likely due to the neutral pH environment present at the intestinal mucosal surface.[75] The mucosal pH rarely strays from neutral and is independent of changes in luminal pH, which could explain why bacteria that are able to quickly attach and invade epithelial cells (e.g., *Salmonella* and *Shigella*) are highly

pathogenic. These bacteria do not solely rely on complex and energy-taxing mechanisms of environmental and host resistance, but simply bypass the bacterial gauntlet in the lumen to hide within the cells.

SCFA Effects on Expression of *spv* Genes *in vitro*

It has been demonstrated that stationary phase growth, heat-shock, nutrient starvation, and energy source depletion are potent inductors of *spv* expression in *Salmonella*.[28;132;187;188] It was recently shown that the specific SCFAs acetate [C2], propionate [C3], butyrate [C4], valerate [C5], and caproate [C6] induce increased *in vitro* expression of *spvR* and *spvB* in *S.* Dublin during log-phase growth at neutral pH.[189] This is important in determining role of the *spv* genes in gastrointestinal pathogenesis, as they are not currently universally accepted to have an enteric-related function. The *spv* genes have been shown to influence the severity of enteritis in a host-adapted model of *S.* Dublin infection in the calf, but this is the only supporting evidence. Examining the response at a lower physiologic pH may be more relevant.

SCFAs and Salmonella Colonization and Infection of Avian Species

A significant amount of research has been performed with regard to preventing salmonella colonization and infection in avian species such as chickens, turkeys, and ducks. Death from infection or salmonella contamination of the eggs or marketable carcass can result in significant lost annual revenue. SCFAs have been studied and used extensively as food additives and therapeutic modalities to minimize the presence of *Salmonella* in layer flocks and hatcheries.[90;190;191] Challenge experiments using broiler chicks colonized with anaerobic cecal flora from adults on day of hatch showed that significantly fewer *Salmonella* were isolated from the digestive tracts of those chicks that were fed 10% lactose as a feed additive. Those chicks also had significantly higher cecal

concentrations of acetic and propionic acids, with lower pHs than controls fed no lactose or a lower concentration.[190] Forced molting induced by feed deprivation in chickens was found to decrease the crop concentrations of acetate, lactate, butyrate and propionate, and significantly sensitize those animals to crop and cecal colonization, with subsequent systemic spread after oral challenge with *S.* Enteriditis.[43] This situation is similar to what is thought to occur in the gastrointestinal tract of the horse after acute dietary changes.

Direct feeding of either carbohydrate or specific SCFAs in avian species has been shown to be protective against salmonella attachment *in vivo*[90] and survival *in vitro*.[192] Diez-Gonzalez et al. demonstrated that increasing amounts of grain fed to cattle significantly increased the SCFA concentrations in the colon approximately fourfold, while rumen concentrations remained unchanged.[181] Annison et al. reported that feeding acetylated, butyrylated, or propionylated starches to rats, preferentially raised the colonic concentrations of those SCFA.[193] Altogether, these results predict tremendous potential for more natural methods of dietary modification to protect against salmonella infection in susceptible livestock and companion animal species.

SCFAs and *Salmonella* in Swine

Salmonella colonization of swine and contamination of meat is an important human health consideration worldwide. The association between SCFAs and resistance to colonization has been studied extensively in the porcine as well as the avian food species. Dietary influence on intestinal pH was shown to be the most important factor in mediating the SCFA-attributed anti-bacterial effects of colon contents in swine at slaughter.[194] This effect was thought due to protonation of the SCFAs into non-ionized forms, as there were no differences in the total levels or relative proportions of SCFAs from two herds with otherwise significantly different shedding levels of *Salmonella*. The

diets were found to contribute significantly to acid-base excretion in the urine as well as colon contents.

SCFAs and Salmonella Colonization and Infection of Bovine Species

The calf model of enteric and systemic salmonellosis has surfaced as a reasonable alternative to *in vitro* or murine models currently available. One advantage to this model is the fact that the bovine species is affected by both host-adapted (Dublin) and broad-host range (e.g., Typhimurium, Anatum, Newport) salmonella serovars. Oral infection of calves with serovar Typhimurium shows the most clinical similarity to salmonella colitis in horses and humans. The spectrum of serovars that affect cattle clinically is very similar to horses (Typhimurium, Typhimurium var. Copenhagen, Anatum, and Newport).[195]

Rumen SCFA mixtures were shown many years ago to be inhibitory to *S.* Typhimurium growth in the presence of a low pH.[196;197] However, a survey of the literature from the dairy and beef industries shows they have minimally advanced their understanding of the effects of SCFA on pathogen growth and survival. Rather they have examined them in an attempt to refine dietary manipulation of lactation[198] or feed efficiency.[199]

Specific Aims

The specific aims of this segment of the study were to:

- Determine the cecal SCFA concentrations, luminal pH, total culturable anaerobic bacterial counts, and protozoal counts of horses before and after treatment with selected antibiotics.
- To examine *spv*+ and *spv*- salmonella isolates in terms of growth rate during anaerobic culture in nutrient broth supplemented with sterile-filtered cecal contents from antibiotic-treated versus non-treated horses.

- To examine *spv*+ and *spv*- salmonella isolates in terms of growth rate during anaerobic culture in nutrient broth adjusted to the mean luminal cecal pH and supplemented with individual SCFAs normally found in horse cecal liquor.

Materials and Methods

IACUC Approval

All protocols, procedures and experiments utilizing experimental animals described in this work were approved by the University of Florida Institutional Animal Care and Use Committee (IACUC) prior to commencement of any experiments.

Subject Coding for Experiments and Data Analysis

Coded reference to individual horses and antibiotic treatments in this document are according to the following legends (Tables 3-5 and 3-6).

Table 3-5. Coding legend for experimental animals

Horse	Letter Code	Number Code
Bill	B	1
Easy	E	2
Scott	S	3
Ted	T	4
Willie	W	5

Surgical Placement of Cecal Cannula in the Horse

A summary of the model and surgical technique using large-bore reinforced-silicone cecal cannulas in horses is included in this document as it has not previously been described in publication. Surgical implantation of a cecal cannula via laparotomy was performed at least one year prior to inclusion in this study. Five thoroughbred geldings, age range 4-15 years were included in the study. The cecum contained a custom, double-flanged, reinforced-silicone cannula (Figure 3-2), surgically placed in the lateral cecal body 30 cm dorsal to the apex and exteriorized through the lower right abdominal wall with 8-12 inches of the cannula visible outside of the horse.

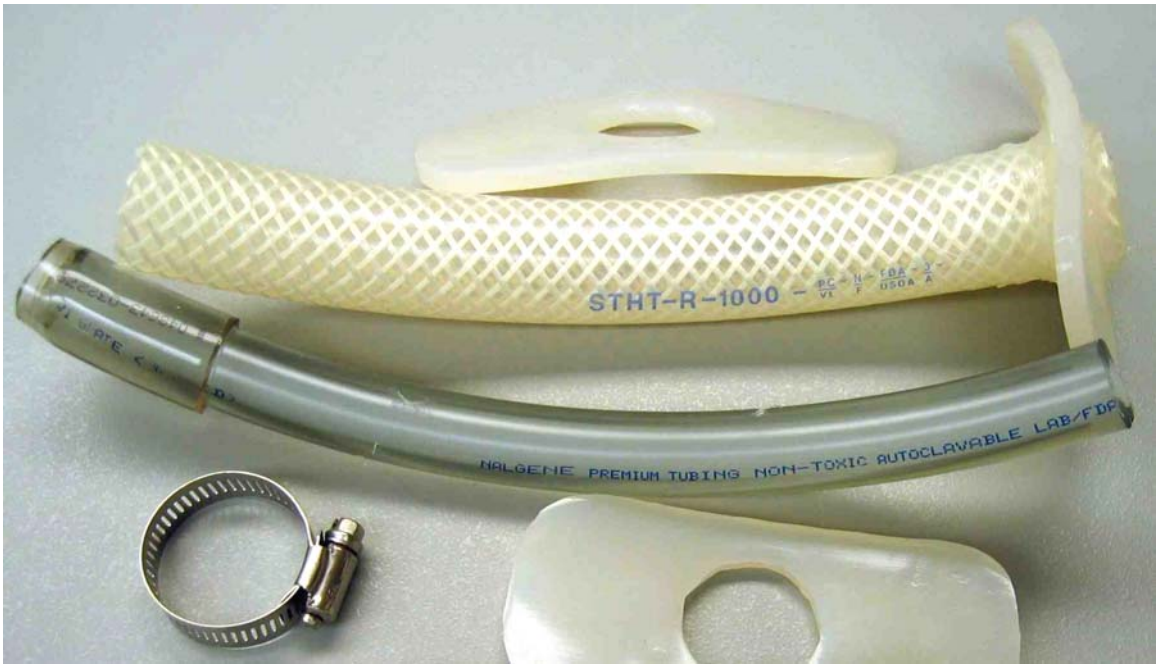

Figure 3-2. Components of indwelling cecal cannula placed into experimental horses. Clockwise from the top: side view of sliding silicone flange placed on the lateral serosal aspect of cecal wall, cannula with fixed silicone interior flange, silicone filled, thick walled PVC tubing used to plug cannula, front view of sliding silicone flange, hose clamp to secure plug within cannula.

The cannula tubing has an outside diameter of 7/8" to 1¼" and an internal diameter of 5/8" to 1", respectively. The cannula was sealed with a tight-fitting silicone plug and secured with a hose clamp to prevent exposure to air, except during collection procedures which were kept to the absolute minimum time necessary. This basic preparation was extremely well tolerated by the horses and has been used by our laboratory with great success for many years as a humane and effective method of repeatedly sampling the cecal lumen with no discomfort to the animal. Photos of an experimental horse and cannula preparation are shown in Figures 3-3 and 3-4. These preparations are not permanent, as time has shown the cannula is slowly expelled via the formation of internal granulation tissue, and the creation of a temporary ceco-cutaneous fistula, which soon closes by second intention.

Figure 3-3. Experimental horse E (2) with cecal cannula 3 years post-implantation.

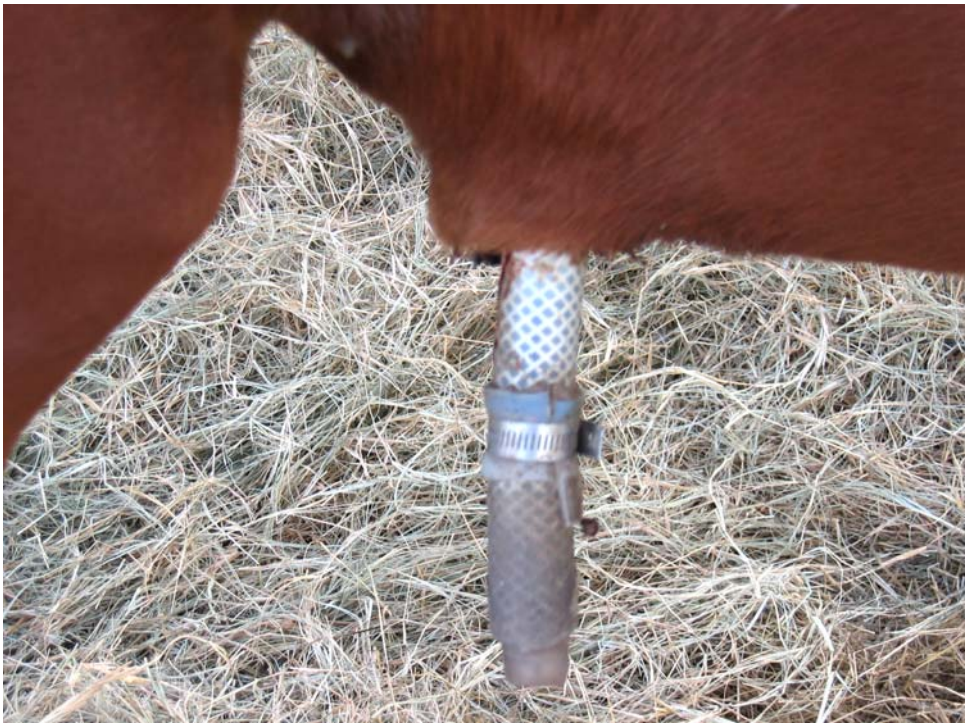

Figure 3-4. Close-up view of cannula *in situ* in experimental horse E (2). Note the formation of a firm swelling intimately associated with the cannula insertion. This is internal granulation tissue forming around the interior silicone flanges which will result in the eventual expulsion of the device.

Antibiotic Treatment of Horses

Each horse received one course of all 4 experimental treatments with no replication, using a randomized block design to minimize effects of treatment order or between horse differences. The horses were weighed once before each treatment to accurately calculate dose rates. Three antibiotics were chosen based on frequency of use in equine medicine, reported relationship regarding antibiotic-associated diarrhea, and typical route of administration. The antibiotic treatments are detailed in Table 3-6.

Table 3-6. Antibiotic treatments of horses

Treatment	**Treatment Code**	**Dose**	**Route of Administration**	**Dosing Interval**
Control (no treatment)	1 - CON	---	---	---
Ceftiofur sodium (Naxcel®)	2 - NAX	2 mg/kg	IM	q 12 h
Oxytetracycline (LA-200®)	3 - TET	10 mg/kg	IV (Diluted into 1 liter NaCl)	q 24 h
Trimethoprim-Sulfamethoxazole	4 - TMPS	30 mg/kg	PO	q 12 h

Ceftiofur sodium is a third-generation cephalosporin with an intermediate spectrum of activity against gram-positive and gram-negative aerobes and some activity against anaerobes. Oxytetracycline is a broad-spectrum agent effective against gram-positive and gram-negative aerobes and anaerobes. Trimethoprim-sulfamethoxazole is a reasonably narrow spectrum potentiated sulfonamide effective against gram-positive and gram-negative aerobes only.[200]

Each antibiotic course was administered for a total of four days twice daily for treatments 2 and 4, and once daily for treatment 3. Four day therapy regimens were decided based on the average amount of time it took for previous investigators to notice derangements in fecal SCFA[49;82-85] and culturable anaerobic fecal flora.[49;86] There was a

minimum of 30 days washout between each treatment for an individual animal, typically between 30 to 45 days.

Equine Cecal Sampling Procedure

Horses were loosely restrained with a halter and lead rope in a stall or in the pasture for sample collection. For each antibiotic treatment, cecal contents were collected and processed within 24 hours prior to commencing therapy and again after three consecutive days of treatment. The time of concentrate feeding and cecal sampling was consistent each day, in order to minimize normal temporal and dietary influences on the cecal SCFA profile.

Several aliquots were quickly collected directly into polypropylene specimen containers via gravity flow (Figure 3-5). The first 100 – 200 ml of contents was allowed to drain before the collection was started to avoid colleting any liquor that had been stagnant in the lumen of the cannula. If the cecal contents would not flow easily or quickly, a 36" stainless steel Chambers catheter was inserted to facilitate sample collection. The sample collection containers were filled to overflow and capped airtight in order to minimize oxygen introduction into the samples. These were immediately transported to the laboratory in a warm water bath. Sample collection via this method attempts to preserve the warm and anaerobic nature of the horse's cecum and the collected sample as much as possible. Individual portions were immediately separated and placed inside an anaerobic chamber for serial dilutions and quantitative culture, kept on the bench for pH measurement, or processed for SCFA analysis. Processing for SCFA analysis involved centrifugation of an 80 ml aliquot of raw cecal contents at 10,000 x *g* and 4°C for 20 min. The supernatant was sterile-filtered through a 0.2 micrometer syringe filter into sterile containers, and the filtered samples were frozen at -80°C for use

as experimental additives to salmonella cultures and SCFA quantification later in the course of the study.

Figure 3-5. Collection of equine cecal contents from indwelling silicone cannula. Note the rapid flow and liquid nature of the contents.

Physical Effects on the Horse

Horses were maintained on grass pasture with twice daily concentrate feeding and fresh water was available at all times. Subjective observations of the experimental horses were made during the treatment periods as well as during washout periods to ensure their well being as well as to monitor for any possible long-term or latent effects of the treatments. Appetite, fecal character and consistency, demeanor, and body condition were observed on at least a weekly basis by investigators, and daily by caregivers.

Effects on Fecal Consistency

Throughout the entire study and specifically during the antibiotic treatment periods, feces were observed for each individual on a daily basis (when available) as the horses were being handled.

Effects on Cecal Content Character

The effect of antibiotic treatment on cecal digesta composition and consistency was evaluated in a subjective manner by one investigator (non-blinded) and was described on the treatment sheets at the time of collection of the pre- or post-treatment sample. Odor, water and fiber content, and color were all described.

Equine Cecal Anaerobe Quantification

Enumeration of anaerobic bacteria in equine cecal contents was carried out according to the method described by Mackie and Wilkins (1988).[166] All sample manipulation was performed inside an anaerobic, climate controlled, combination chamber and incubator with a 10% CO_2 - 85% N_2 - 5% H_2 atmosphere (Bactron™ 1.5, Sheldon Manufacturing, Cornelius, OR). Sealed aliquots of the freshly collected cecal contents were placed into the chamber as soon as possible after collection (within 15 min). Each sample was serially diluted from 10^{-1} to 10^{-10} in pre-reduced 0.9% sterile phosphate-buffered saline (PBS) and plated onto commercially manufactured pre-reduced anaerobically sterilized (PRAS) media, Brucella blood agar containing 75 micrograms/ml of gentamicin sulfate (AS-141G, Anaerobe Systems, San Jose, CA). CFU/ml of cecal contents was estimated according to the Miles-Misra technique for quantification of viable bacteria. A 20 microliter drop of each dilution was applied to the plate surface, allowed to dry, and incubated within the anaerobic chamber with plates inverted at 37°C. Samples were replicate plated four times for average estimation of CFU/ml. Colony

counts of anaerobic flora were performed after one and four days of incubation and reported in CFU/ml of cecal contents. No attempt was made to identify or classify the bacteria, only quantify.

pH Analysis of Equine Cecal Contents

Aliquots of the freshly collected cecal contents were measured for pH as soon as possible after collection (within 15 min). The mean of three different measurements was taken for the sample value. pH was measured using a digital desktop pH meter (Corning Inc. Life Sciences, Acton, MA) which was calibrated using two standard buffers (pH 7.0 and pH 10.0) before each sample measurement. The lowest mean postprandial cecal pH values in horses were obtained approximately 4-7 h after a meal[168] and sampling time in these experimental horses was adjusted to occur within this window.

Short-Chain Fatty Acid Analysis of Equine Cecal Contents

Samples of the ultra-centrifuged and filtered cecal supernatant were frozen at -80°C until analysis. Thawed supernatant SCFA composition was measured using capillary glass chromatography (Autosystem II, Perkin Elmer, Boston, MA) with splitless automatic injection onto a Nukol Fused Silica Capillary Column, 30 mm x 0.25 mm ID (Supelco Chromatography, Bellefonte, PA) and using helium as the carrier gas. SCFAs were detected using a flame ionization detector and peaks were integrated and compared with external standards using the Turbochrome 3 integration computer software (Perkin Elmer, Boston, MA). Previous experiments with cecal contents from the same horses had shown that optimum conditions for separation were obtained with the temperatures set at 250°C for the injector, 160°C for the oven, and 180°C for the detector. Acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and ethyl butyrate were identified.

Protozoal Quantification of Cecal Contents from Horses Treated with Antibiotics

Protozoal counts were performed according to the method described by Adam.[201] A 1 ml sample of freshly collected cecal contents was added to a 2 ml microcentrifuge tube containing 1 ml of buffered 10% formalin. The samples were stored at 2-8°C until analysis. After thawing, 500 microliters of the formalin diluted sample was added to 150 microliters of 0.5% methyl green in 7% acetic acid solution. The sample was vortexed and added to the counting well of a McMaster slide. A 0.15 ml aliquot of diluted sample was examined for the presence of protozoa. Counts were normalized to number of protozoa per ml of cecal contents, and statistically compared across horses, times, and treatments.

***In vitro* Short-Chain Fatty Acid Growth Comparison**

Three different SCFAs were compared for their *in vitro* ability to affect growth rates of *Salmonella* in an anaerobic environment. Acetate, butyrate, and propionate were chosen based on their natural predominance in the mammalian cecum and large intestine. Inhibitory as well as stimulatory growth was investigated using a nutritionally robust (LB) or minimal media (M9) respectively. The sodium salts of acetic acid, butyric acid or propionic acid (Sigma-Aldrich, St. Louis, MO) were added to pre-reduced broth media in an anaerobic chamber at both 30mM and 100mM concentrations. The pH was adjusted to 6.5 in all experimental solutions, equivalent to the mean cecal pH of the un-treated experimental horses. NaCl at 30mM and 100mM was added to control tubes as an isosmolar equivalent. χ3306 was compared with χ3337 for all experiments to examine the effects of virulence plasmid and *spv* gene presence on growth-rate. Standing overnight cultures of each isolate were diluted to approximately 1×10^{-3} to 1×10^{-5} in the experimental tubes, and the cells were grown for 10-12 h. Aliquots were taken at time 0,

2 h, 4 h, 6 h, 8 h, 10 h, and ±12 h and serially diluted and plated on LB agar to enumerate CFU/ml. Experiments were repeated to validate results. Growth curves were produced from these data and compared statistically with SCFA and concentration as factors.

Since the two *S.* Typhimurium strains exhibited nearly identical shape in their respective growth curve responses (regardless of initial bacterial concentrations, which may have been different) to the control and short-chain fatty acid solutions, the data were combined and they were treated as replicates instead of individual experiments. All experimental solutions were prepared to pH 6.5, which approximates the measured luminal pH of the cecum in horses.[1]

The concentrations chosen for the experimental SCFA solutions in this study were above the normal physiologic range for mammalian large intestine. They were chosen based on similar studies in other species and genera, as well as the food protection industry.[158;175;184;185;202] This was decided in order to observe (in the smallest number of experiments possible) whether an effect on anaerobic growth rates existed for the compounds. Continued examination with titration down towards the physiologic range would yield the *in vitro* breakpoint inhibitory concentration, and this could be supplemented with information obtained from growth in a variety of other media—including raw intestinal contents spiked with SCFA. Newer technologies allow quantification of specific bacteria within a heterogeneous bacterial suspension.[203]

In vitro Effects of Cecal Liquor from Antibiotic-treated Horses on Anaerobic Growth of *Salmonella*

The effect of adding filter-sterilized cecal contents, from horses treated with selected antibiotics as compared to untreated controls on anaerobic growth of *Salmonella*

[1] Data from this work estimated the mean luminal pH in the cecum of horses to be 6.6

in vitro, was performed according to a modification of the method described in mice by Que.[80] Cecal contents were collected from horses and processed as described previously—24 h prior to initiation of antibiotic treatment and at the conclusion. The frozen aliquots of cecal liquor were thawed at 4°C overnight and either pooled per treatment group or added individually at 10% (v/v) concentration to both pre-reduced complete M9 with glucose or pre-reduced LB broth in an anaerobic chamber (Bactron™ 1.5, Sheldon Manufacturing, Cornelius, OR). Static overnight cultures of salmonella strains χ3306 and χ3337 were added to the experimental tubes at starting dilutions ranging from 1 x 10^{-3} to1 x 10^{-5}. Aliquots were taken at time 0, 2 h, 4 h, 6 h, 8 h, 10 h, and ±12 h, serially diluted in PBS, and plated to enumerate CFU/ml. The filter-sterilized cecal contents from the five experimental horses were pooled by treatment for the preliminary experiments and then examined by individual horse at the conclusion. The growth of *S.* Typhimurium strain χ3306 was also compared to χ3337 to examine if any effect of the virulence plasmid with *spv* genes could be determined. Growth was examined in both a nutritionally rich medium and M9 minimal medium with glucose to evaluate the possibility of inhibitory as well as trophic effects. The filter-sterilized cecal contents from antibiotic-treated horses were added in 10% concentrations for all experiments. Growth curves were produced from these data and compared statistically, with horse and treatment as factors. Based on preliminary results from both pooled additive experiments, it was decided to do the remaining individual horse experiments in nutritionally limited M9 minimal medium to examine for potential trophic effects of the additives.

pH was measured of the stored liquor samples before the addition experiments and was found to correlate with original measured pH values. It was decided that these values would not be normalized prior to the *in vitro* experiments, but remain in their original state to more closely approximate physiologic conditions. If an effect was determined from the unadulterated samples, the experiment would be repeated with the pH values neutralized to determine if the effect was due to pH alone.

Statistical Methods

Data from the total culturable anaerobe counts, pH of cecal contents, and protozoal counts were analyzed non-parametrically using Wilcoxons Sign Rank Test for two related values in a commercial statistical analysis program (SPSS for Windows version 11.0, SPSS, Chicago, IL). Logarithmic transformations were performed on all bacterial count data before analysis. The cecal fluid addition and SCFA addition experiments were analyzed using analysis of variance with repeated measures. Significance levels were set at $p=0.05$ for all experimental data.

Results

Effects on the Horse

All animals successfully completed all treatments during the course of the study, with no complications or observed side-effects. Fecal consistency remained normal for all horses during the treatment periods, and there was no apparent softening (to "cow pie" consistency) or loss of shape from the normal fecal ball appearance. There was no observable effect to unbiased individuals of antibiotic treatment or placebo on fecal character or consistency for any horse during any experiment. No changes were reported by caretakers regarding the horses' appetite during the antibiotic trial periods. Body weight and condition scores remained constant throughout the trial period.

Cecal contents tended to be thicker (oatmeal consistency), bright green (depending on the amount of pasture available at the time of the year), with an increased visible fiber content, before any of the treatments (i.e., first treatment pre-treatment sample for each horse). It was noted that this normally thick consistency tended to become more watery, darker colored tending toward brown, with less particulate matter, after an experimental treatment, especially the oxytetracycline treatment. The odor of the contents also changed, from the characteristic sharp, acrid odor with a hint of background sweetness, to a distinctly unpleasant or foul smell. Though the fact that a horse was being treated with antibiotics vs. the control treatment (for all three drugs tested) could be determined by observing the cecal contents, it was not discernable by evaluating the horse, its demeanor, or its feces.

Effects on Cecal pH

The baseline (pre-treatment) pH values measured for equine cecal contents were in agreement with other investigators (mean of 6.6).[155;166;170;204] There was no statistically significant effect of treatment on pH of the cecal liquor for any of the antibiotics studied as compared to the control treatment. Data from all horses and all treatments with means is summarized in Tables 3-7 through 3-10. An increase in cecal liquor pH was only seen with the oxytetracycline treatment and it was not significantly different than the baseline range. Measured pH values during treatment periods were also within the published ranges of normal horses.

Table 3-7. Cecal liquor pH of cannulated horses before and after 4 days of control (no) antibiotic treatment

HORSE		PRE	POST		CHANGE	MEAN PRE
1		6.10	6.08		-0.02	6.34
2		6.26	6.09		-0.18	**MEAN POST**
3		6.17	5.74		-0.43	6.10
4		6.21	6.11		-0.10	**MEAN CHANGE**
5		6.94	6.49		-0.45	-0.24

Table 3-8. Cecal liquor pH of cannulated horses treated with intramuscular ceftiofur sodium at 2 mg/kg twice daily, before and after 4 days of treatment

HORSE		PRE	POST		CHANGE	MEAN PRE
1		6.56	6.68		0.12	6.74
2		6.56	7.00		0.44	**MEAN POST**
3		6.65	6.61		-0.05	6.84
4		6.80	6.74		-0.06	**MEAN CHANGE**
5		7.13	7.15		0.02	0.09

Table 3-9. Cecal liquor pH of cannulated horses treated with intravenous oxytetracycline at 10 mg/kg once daily, before and after 4 days of treatment

HORSE		PRE	POST		CHANGE	MEAN PRE
1		6.33	6.40		0.07	6.61
2		6.74	7.15		0.41	**MEAN POST**
3		6.86	7.07		0.22	6.92
4		6.41	6.98		0.57	**MEAN CHANGE**
5		6.74	7.00		0.26	0.31

Table 3-10. Cecal liquor pH of cannulated horses treated with oral trimethoprim-sulfamethoxazole at 30 mg/kg twice daily, before and after 4 days of treatment

HORSE		PRE	POST		CHANGE	MEAN PRE
1		6.86	6.37		-0.49	6.73
2		6.56	6.17		-0.39	**MEAN POST**
3		6.31	5.97		-0.34	6.39
4		7.47	6.82		-0.65	**MEAN CHANGE**
5		6.43	6.63		0.21	-0.33

Effects on Cecal Protozoal Counts

Results are shown in Tables 3-11 through 3-14. In each experiment there appears to be one outlier in terms of pre-treatment protozoal counts or response to the treatments, though it was not always the same horse.

Table 3-11. Total protozoal counts per ml of cecal contents from cannulated horses before and after 4 days of control (no) antibiotic treatment

Horse	PRE	POST	Absolute Change	% Change
1	966	2984	2018	208.9
2	1666	884	-782	-46.9
3	1634	516	-1118	-68.4
4	2066	2250	184	8.9
5	1334	8100	6766	507.2
MEAN	**1533**	**2947**	**↑ 1414**	

Table 3-12. Total protozoal counts per ml of cecal contents from cannulated horses treated with intramuscular ceftiofur sodium at 2 mg/kg twice daily, before and after 4 days of treatment

Horse	PRE	POST	Absolute Change	% Change
1	7266	4766	-2500	-34.4
2	1534	4966	3432	223.7
3	1616	4534	2918	180.6
4	7884	4916	-2968	-37.7
5	11950	9500	-2450	-20.5
MEAN	**6050**	**5736**	**↓ 314**	

Table 3-13. Total protozoal counts per ml of cecal contents from cannulated horses treated with intravenous oxytetracycline at 10 mg/kg once daily, before and after 4 days of treatment

Horse	PRE	POST	Absolute Change	% Change
1	850	1966	1116	131.3
2	2584	3400	816	31.6
3	8834	10934	2100	23.8
4	716	6566	5850	817.0
5	10150	6934	-3216	-31.7
MEAN	**4627**	**5960**	**↑ 1333**	

Table 3-14. Total protozoal counts per ml of cecal contents from cannulated horses treated with oral trimethoprim-sulfamethoxazole at 30 mg/kg twice daily, before and after 4 days of treatment

Horse	PRE	POST	Absolute Change	% Change
1	3884	2800	-1084	-27.9
2	616	5684	5068	822.7
3	3838	1900	-1938	-50.5
4	4700	2684	-2016	-42.9
5	8566	13650	5084	59.4
MEAN	**4321**	**5344**	**↑ 1023**	

Both the percent change and the absolute change were examined to account for initial between horse differences in the numbers of protozoa. There were no statistically significant differences in either the absolute means or the percentage change means compared to the control treatment for any antibiotic treatment (p=0.500 to 0.893). One outlying individual was removed from the analyses and the results (no significant effect of treatments) were similar. Values obtained for pre and post-treatment means were all within the range of published values for equine cecal protozoal organisms.

Effects on Cecal SCFA Quantities and Proportions

The effects of antibiotic treatment on equine cecal SCFA profiles are detailed in Tables 3-15 through 3-18. There were no significant differences in the absolute concentration of total SCFAs measured for any of the antibiotic treatments (as compared to the control treatment), but there were significant differences in specific individual SCFA concentrations for all three antibiotic treatments. The individual SCFAs were examined using the percent change from pre-treatment values compared to the control treatment. Several individual acids for different treatments were significant or approached significance. For the ceftiofur treatment—acetate decreased p=0.080, for the trimethoprim-sulfamethoxazole treatment—acetate increased p=0.080 and isovalerate decreased p=0.043, and for the oxytetracycline treatment—propionate increased p=0.043, isobutyrate increased p=0.043, butyrate increased p=0.043, isovalerate increased p=0.043, and valerate increased p=0.080. Total SCFA percentage change values approached significance in the ceftiofur (p=0.080) and trimethoprim-sulfamethoxazole (p=0.080) groups when compared to the control group percentage change.

Table 3-15. Cecal liquor concentrations of individual and total SCFAs from cannulated horses, before and after 4 days of control (no) treatment

Horse	Treatment	Time	A	P	IB	B	IVA	V	EB	TOTAL
			Concentration in mM							
1	1	pre	5.04	6.67	9.94	7.85	2.57	8.01	5.96	**46.04**
1	1	post	6.30	5.87	9.03	8.73	3.32	10.15	9.33	**52.72**
2	1	pre	4.61	6.34	6.19	15.86	2.85	11.27	7.21	**54.34**
2	1	post	4.44	5.97	6.39	13.72	3.64	8.81	10.04	**53.01**
3	1	pre	3.47	1.35	4.18	3.41	2.53	2.27	11.64	**28.85**
3	1	post	4.30	2.70	6.13	6.18	3.24	3.86	10.78	**37.19**
4	1	pre	3.84	2.15	4.49	4.82	3.02	2.93	9.70	**30.95**
4	1	post	5.77	4.16	7.42	7.73	4.46	4.63	8.61	**42.78**
5	1	pre	3.20	1.63	4.55	3.41	3.22	2.53	8.27	**26.81**
5	1	post	4.04	2.72	5.57	5.46	3.33	4.14	9.31	**34.57**
		MEAN PRE	4.03	4.03	3.63	5.87	7.07	2.84	5.40	8.55
		MEAN POST	4.97	4.97	4.28	6.91	8.36	3.60	6.32	9.61
		% Change of Mean	**↑23.17%**	**↑18.06%**	**↑17.68%**	**↑18.29%**	**↑26.84%**	**↑16.97%**	**↑12.39%**	**↑17.81%**
		Mean of % Changes	**↑24.35%**	**↑48.51%**	**↑25.68%**	**↑39.89%**	**↑27.21%**	**↑39.32%**	**↑17.95%**	**↑21.64%**

A = acetate, P = propionate, IB = isobutyrate, B = butyrate, IVA = isovalerate, V = valerate, EB = ethyl butyrate

Table 3-16. Cecal liquor concentrations of individual and total SCFAs from cannulated horses treated with intramuscular ceftiofur sodium at 2 mg/kg twice daily, before and after 4 days of treatment

Horse	Treatment	Time	A	P	IB	B	IVA	V	EB	TOTAL
			Concentration in mM							
1	2	pre	6.67	5.62	8.39	12.55	3.72	11.41	9.30	**57.66**
1	2	post	6.11	4.39	6.70	11.14	3.14	7.99	8.62	**48.09**
2	2	pre	4.75	7.52	6.40	15.07	2.99	9.88	17.09	**63.69**
2	2	post	4.34	5.84	5.94	12.08	3.69	8.35	7.12	**47.36**
3	2	pre	4.83	3.01	6.83	6.04	4.10	4.00	7.02	**35.84**
3	2	post	4.85	5.09	8.31	10.65	4.50	6.40	7.91	**47.70**
4	2	pre	4.38	5.60	7.22	10.96	3.83	6.49	8.49	**46.98**
4	2	post	4.84	7.15	8.10	13.88	5.04	8.11	9.47	**56.58**
5	2	pre	4.48	4.73	7.40	9.56	4.96	5.79	9.60	**46.51**
5	2	post	4.51	4.49	6.93	6.91	3.59	4.08	8.81	**39.32**
		MEAN PRE	5.02	5.02	5.30	7.25	10.83	3.92	7.51	10.30
		MEAN POST	4.93	4.93	5.39	7.19	10.93	3.99	6.99	8.39
		% Change of Mean	**↓1.84%**	**↑1.81%**	**↓0.75%**	**↑0.89%**	**↑1.80%**	**↓7.02%**	**↓18.57%**	**↓4.63%**
		Mean of % Changes	**↑2.40%**	**↑28.34%**	**↑6.93%**	**↑33.64%**	**↑11.11%**	**↑21.64%**	**↑7.35%**	**↓0.818%**
		p value (compared to control)	**0.080**	**0.686**	**0.345**	**0.893**	**0.225**	**0.345**	**0.686**	**0.080**

A = acetate, P = propionate, IB = isobutyrate, B = butyrate, IVA = isovalerate, V = valerate, EB = ethyl butyrate

Table 3-17. Cecal liquor concentrations of individual and total SCFAs from cannulated horses treated with intravenous oxytetracycline at 10 mg/kg once daily, before and after 4 days of treatment

Horse	Treatment	Time	A	P	IB	B	IVA	V	EB	TOTAL
			Concentration in mM							
1	3	pre	5.86	4.73	8.10	11.31	3.78	8.48	7.71	**49.96**
1	3	post	5.12	3.68	5.09	8.40	2.70	7.98	8.67	**41.65**
2	3	pre	4.29	8.40	6.59	16.41	3.23	10.38	7.72	**57.02**
2	3	post	4.78	8.65	7.71	17.02	4.43	10.26	6.56	**59.42**
3	3	pre	4.67	4.55	7.40	9.24	3.56	6.00	9.27	**44.71**
3	3	post	4.52	5.12	8.14	11.00	5.32	6.37	7.28	**47.75**
4	3	pre	4.09	3.28	6.12	7.02	3.14	4.20	8.39	**36.23**
4	3	post	4.46	4.79	7.35	9.33	4.02	5.97	6.84	**42.78**
5	3	pre	4.09	1.86	5.38	3.51	3.63	3.10	7.72	**29.30**
5	3	post	4.16	4.64	6.38	8.81	3.44	5.73	6.85	**40.01**
		MEAN PRE	4.60	4.60	4.56	6.72	9.50	3.47	6.43	8.16
		MEAN POST	4.61	4.61	5.38	6.94	10.91	3.98	7.26	7.24
		% Change of Mean	**↑0.19%**	**↑17.87%**	**↑3.19%**	**↑14.91%**	**↑14.78%**	**↑12.89%**	**↓11.28%**	**↑6.62%**
		Mean of % Changes	**↓1.09%**	**↑9.50%**	**↑0.04%**	**↑8.83%**	**↑4.31%**	**↑1.99%**	**↓9.93%**	**↑6.49%**
		p value (compared to control)	**0.043**	**0.043**	**0.043**	**0.043**	**0.043**	**0.080**	**0.345**	**0.138**

A = acetate, P = propionate, IB = isobutyrate, B = butyrate, IVA = isovalerate, V = valerate, EB = ethyl butyrate

Table 3-18. Cecal liquor concentrations of individual and total SCFAs from cannulated horses treated with oral trimethoprim-sulfamethoxazole at 30 mg/kg twice daily, before and after 4 days of treatment

Horse	Treatment	Time	A	P	IB	B	IVA	V	EB	TOTAL
			Concentration in mM							
1	4	pre	3.41	5.50	4.74	12.40	2.55	9.56	7.90	**46.06**
1	4	post	4.76	6.09	7.86	12.26	3.89	10.67	11.00	**56.52**
2	4	pre	3.74	3.76	5.85	9.33	3.77	5.57	7.40	**39.42**
2	4	post	4.18	2.46	4.83	5.45	2.84	4.13	10.16	**34.04**
3	4	pre	5.49	4.89	9.00	9.86	6.50	5.46	5.28	**46.48**
3	4	post	4.47	3.79	7.27	6.57	4.58	5.21	7.41	**39.29**
4	4	pre	4.66	4.39	7.42	8.94	4.30	5.56	9.85	**45.14**
4	4	post	4.49	2.49	5.48	4.93	3.66	3.80	10.87	**35.72**
5	4	pre	4.23	3.64	6.02	6.90	3.19	5.04	7.76	**36.78**
5	4	post	4.30	4.10	6.52	8.00	4.41	4.37	6.99	**38.69**
	MEAN PRE		4.30	4.30	4.44	6.61	9.49	4.06	6.24	7.64
	MEAN POST		4.44	4.44	3.79	6.39	7.44	3.87	5.64	9.29
	% Change of Mean		**↑3.15%**	**↓14.71%**	**↓3.26%**	**↓21.55%**	**↓4.61%**	**↓9.68%**	**↑21.56%**	**↓4.49%**
	Mean of % Changes		**↓6.75%**	**↓14.35%**	**↓5.18%**	**↓14.90%**	**↓9.91%**	**↓9.61%**	**↓1.74%**	**↓7.38%**
	p value (compared to control)		**0.080**	**0.225**	**0.225**	**0.138**	**0.043**	**0.138**	**0.138**	**0.080**

A = acetate, P = propionate, IB = isobutyrate, B = butyrate, IVA = isovalerate, V = valerate, EB = ethyl butyrate

Effects on Cecal Anaerobic Bacteria

The results of experiments examining the effects of antibiotic treatment on culturable cecal anaerobes are detailed in Tables 3-19 through 3-22. Unfortunately, due to equipment malfunction, cecal anaerobe counts from only one horse in the control group were available. All other treatment groups had values for all five horses in them. This deficiency of data in the control group makes statistical comparison difficult, as one individual is being used for comparison of all treatments. Despite the missing information, some trends were evident (Figure 3-6). No significance was obtained with any treatment percentage change from pre-treatment values compared to the control horse ($p=0.138$ to 0.500). Subjectively, there was a trend for the TMPS treatment to decrease the mean counts for all horses from pre-treatment values as compared to the NAX and TET treatments which did not show any obvious trend. The pre-treatment counts for total anaerobes also were much higher in all treatments compared to the control; however, this may be due to the influence of only one individual in the control group thus making comparison inaccurate.

Table 3-19. Mean counts of culturable anaerobic bacteria from serial dilutions of raw equine cecal liquor, from 5 cannulated horses, before and after 4 days of control (no) treatment. The dilution shaded in green was chosen for comparison.

Dilution	**Control PRE**		**Control POST**	
10^{-1}	TNTC	n=1	TNTC	n=1
10^{-2}	TNTC	n=1	TNTC	n=1
10^{-3}	20.5	n=1	47.8	n=1
10^{-4}	5.5	n=1	8.3	n=1
10^{-5}	0.5	n=1	1.8	n=1
10^{-6}	0	n=1	0	n=1
10^{-7}	0	n=1	0	n=1
10^{-8}	0	n=1	0	n=1

Table 3-20. Mean counts of culturable anaerobic bacteria from serial dilutions of raw equine cecal liquor, from 5 cannulated horses treated with intramuscular ceftiofur sodium at 2 mg/kg twice daily, before and after 4 days of treatment. The dilution shaded in green was chosen for comparison.

Dilution	Ceftiofur PRE		Ceftiofur POST	
10^{-1}	TNTC	n=5	TNTC	n=5
10^{-2}	TNTC	n=5	TNTC	n=5
10^{-3}	40	n=3	57.1	n=3
10^{-4}	14.8	n=5	17.9	n=5
10^{-5}	2	n=5	1.9	n=5
10^{-6}	0.2	n=5	0.2	n=5
10^{-7}	0	n=5	0.1	n=5
10^{-8}	0	n=5	0	n=5

Table 3-21. Mean counts of culturable anaerobic bacteria from serial dilutions of raw equine cecal liquor, from 5 cannulated horses treated with intravenous oxytetracycline at 10 mg/kg once daily, before and after 4 days of treatment. The dilution shaded in green was chosen for comparison.

Dilution	Oxytetracycline PRE		Oxytetracycline POST	
10^{-1}	TNTC	n=5	TNTC	n=5
10^{-2}	TNTC	n=5	TNTC	n=5
10^{-3}	35.6	n=4	17.8	n=3
10^{-4}	7.9	n=5	10.8	n=5
10^{-5}	1.1	n=5	1.2	n=5
10^{-6}	0	n=5	0.1	n=5
10^{-7}	0	n=5	0	n=5
10^{-8}	0	n=5	0	n=5

Table 3-22. Mean counts of culturable anaerobic bacteria from serial dilutions of raw equine cecal liquor, from cannulated horses treated with oral trimethoprim-sulfamethoxazole at 30 mg/kg twice daily, before and after 4 days of treatment. The dilution shaded in green was chosen for comparison.

Dilution	Trimethoprim-Sulfa PRE		Trimethoprim-Sulfa POST	
10^{-1}	TNTC	n=5	TNTC	n=5
10^{-2}	TNTC	n=5	107.5	n=1
10^{-3}	80.6	n=5	26.3	n=4
10^{-4}	16.7	n=5	16.8	n=5
10^{-5}	1.1	n=5	2.4	n=5
10^{-6}	0.4	n=5	0.5	n=5
10^{-7}	0	n=5	0	n=5
10^{-8}	0	n=5	0	n=5

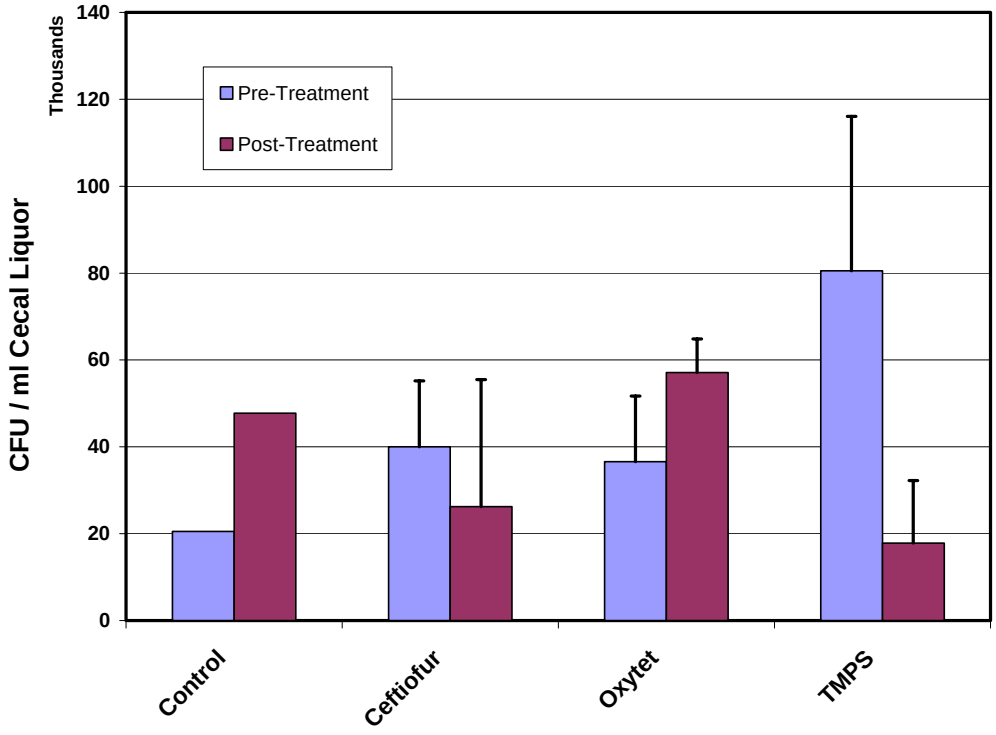

Figure 3-6. Mean cecal anaerobic culture counts expressed as CFU / ml of liquor from five horses before and after treatment with control (no treatment), ceftiofur, oxytetracycline, or trimethoprim-sulfamethoxazole.

In vitro Effects of SCFAs on Anaerobic Growth of *Salmonella*

Figures 3-7 through 3-10 demonstrate no significant differences between the plasmid and *spv* gene containing strain χ3306 versus the same plasmid cured (and *spv* deficient) strain χ3337. Data for all short-chain fatty acid solution experiments is expressed as log CFU/ml with 95%CI error bars.

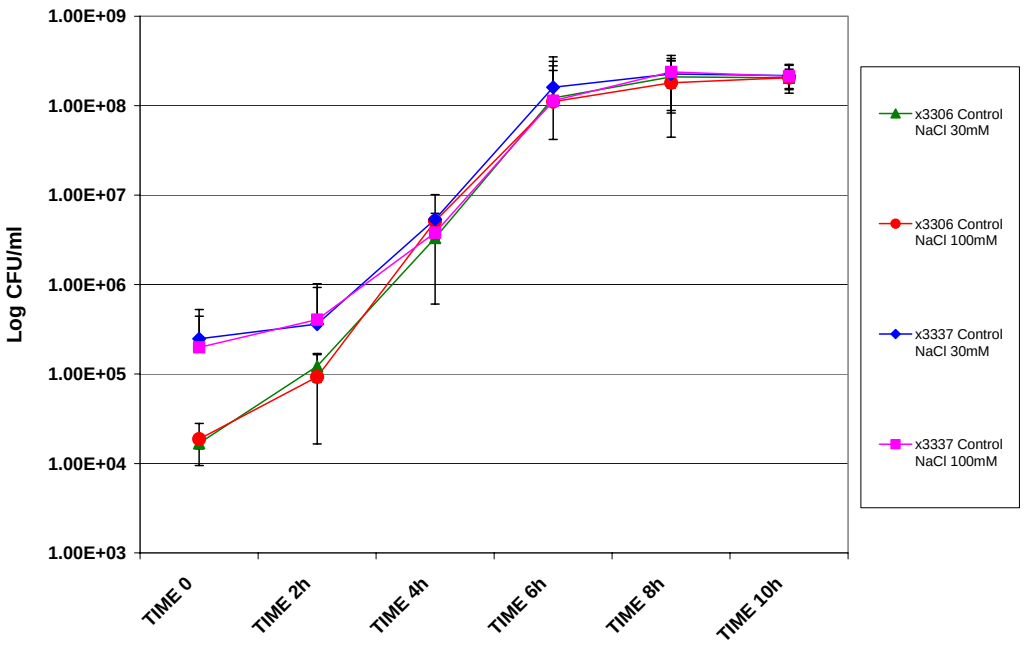

Figure 3-7. The effect of LB broth with sodium chloride (control treatment) added at 30mM or 100mM on anaerobic growth of *S.* Typhimurium χ3306 vs. χ3337. All solutions were pH 6.5. Error bars represent 95%CI for two replicates of the experiment.

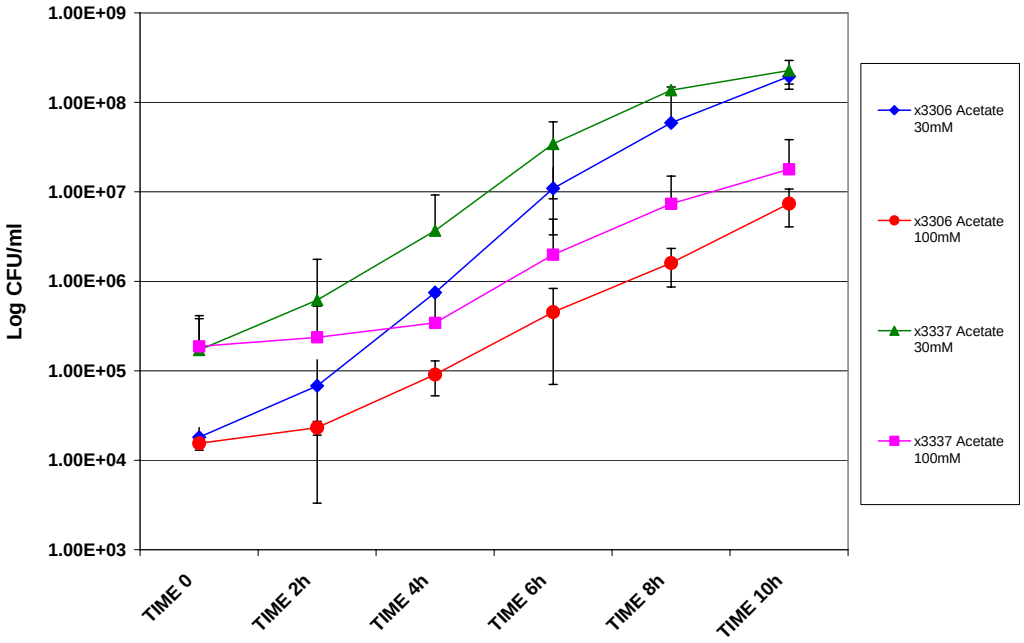

Figure 3-8. The effect of LB broth with sodium acetate added at 30mM or 100mM on anaerobic growth of *S.* Typhimurium χ3306 vs. χ3337. All solutions were pH 6.5. Error bars represent 95%CI for two replicates of the experiment.

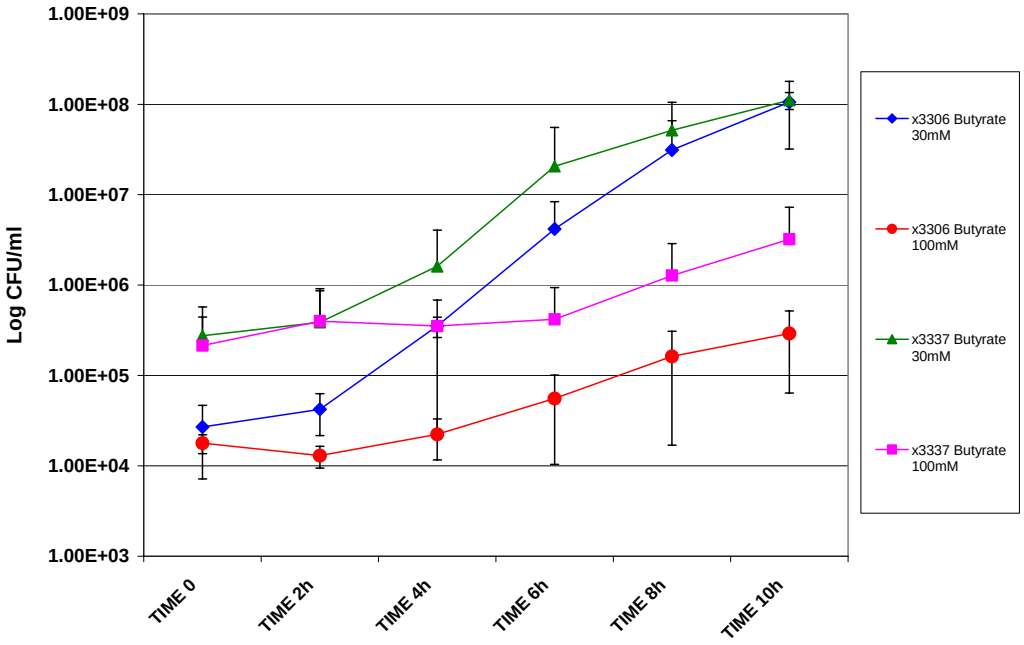

Figure 3-9. The effect of LB broth with sodium butyrate added at 30mM or 100mM on anaerobic growth of *S.* Typhimurium χ3306 vs. χ3337. All solutions were pH 6.5. Error bars represent 95%CI for two replicates of the experiment.

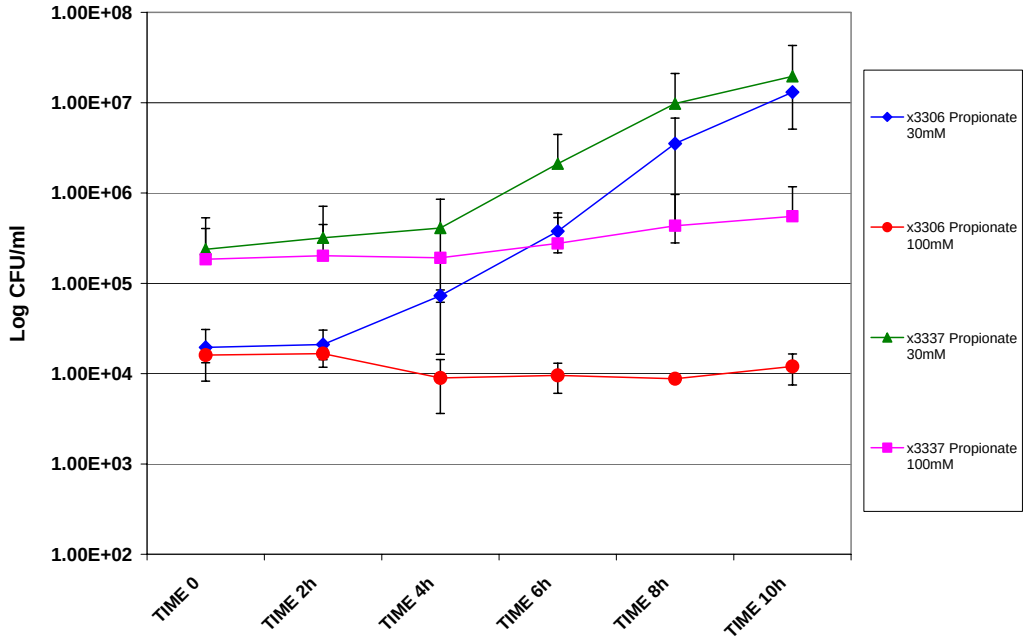

Figure 3-10. The effect of LB broth with sodium propionate added at 30mM or 100mM on anaerobic growth of *S.* Typhimurium χ3306 vs. χ3337. All solutions were pH 6.5. Error bars represent 95%CI for two replicates of the experiment.

Effect of Acetate

There was a dose-dependent inhibitory effect of acetate on the anaerobic growth of *Salmonella* in a nutritionally rich medium (LB broth). Figure 3-11 shows the growth curves of *S.* Typhimurium in LB broth with 30 or 100mM acetate solutions as compared to equiosmolar NaCl control solutions. After 10 h of growth, the levels of bacteria growing in the 100mM acetate solution were a full log fewer than those growing in either the 30 or 100mM NaCl, or the 30mM acetate solution; however, they were still growing. The 30mM acetate solution did not inhibit *Salmonella* significantly until the 6 h time point, and these differences were no longer significant at the 10 h time point.

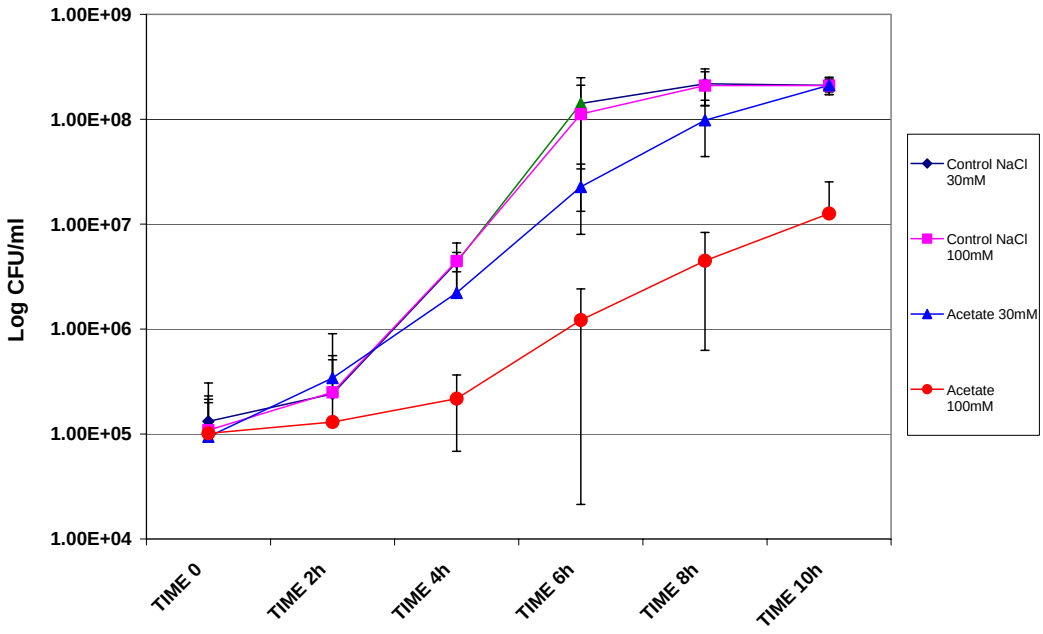

Figure 3-11. The effect of LB broth with sodium acetate at 30mM or 100mM compared to NaCl at 30mM or 100mM on anaerobic growth of *S.* Typhimurium. All solutions were pH 6.5. Error bars represent 95%CI for four replicates of the experiment.

Effect of the Plasmid and *spv* Genes on Acetate Response

There was no statistically significant effect of the presence of the virulence plasmid with the *spv* genes on the growth of *Salmonella* in the presence of acetate at 30mM or

100mM concentrations. Dissimilar bacterial starting concentrations between the two experiments made the growth curves appear different.

Effect of Butyrate

There was a dose-dependent inhibitory effect of butyrate on the anaerobic growth of *Salmonella* in a nutritionally rich medium (LB broth). Figure 3-12 shows the growth curves of *S.* Typhimurium in LB broth with 30 or 100mM butyrate solutions as compared to equiosmolar NaCl control solutions.

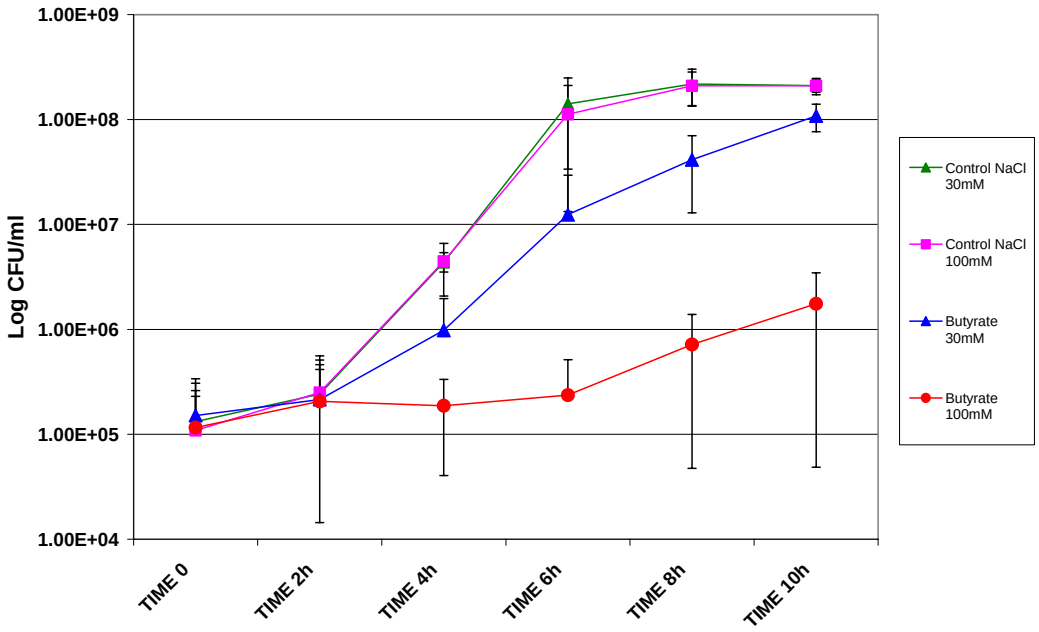

Figure 3-12. The effect of LB broth with sodium butyrate at 30mM or 100mM compared to NaCl at 30mM or 100mM on anaerobic growth of *S.* Typhimurium. All solutions were pH 6.5. Error bars represent 95%CI for four replicates of the experiment.

Effect of the Plasmid and *spv* Genes on Butyrate Response

There was no statistically significant effect of the presence of the virulence plasmid with the *spv* genes on the growth of *Salmonella* in the presence of butyrate at 30mM or 100mM concentrations. Dissimilar bacterial starting concentrations between the two experiments made the growth curves appear different.

Effect of Propionate

There was a dose-dependent inhibitory effect of propionate on the anaerobic growth of *Salmonella* in a nutritionally rich medium (LB broth). Figure 3-13 shows the growth curves of *S.* Typhimurium in LB broth with 30 or 100mM propionate solutions as compared to equiosmolar NaCl control solutions.

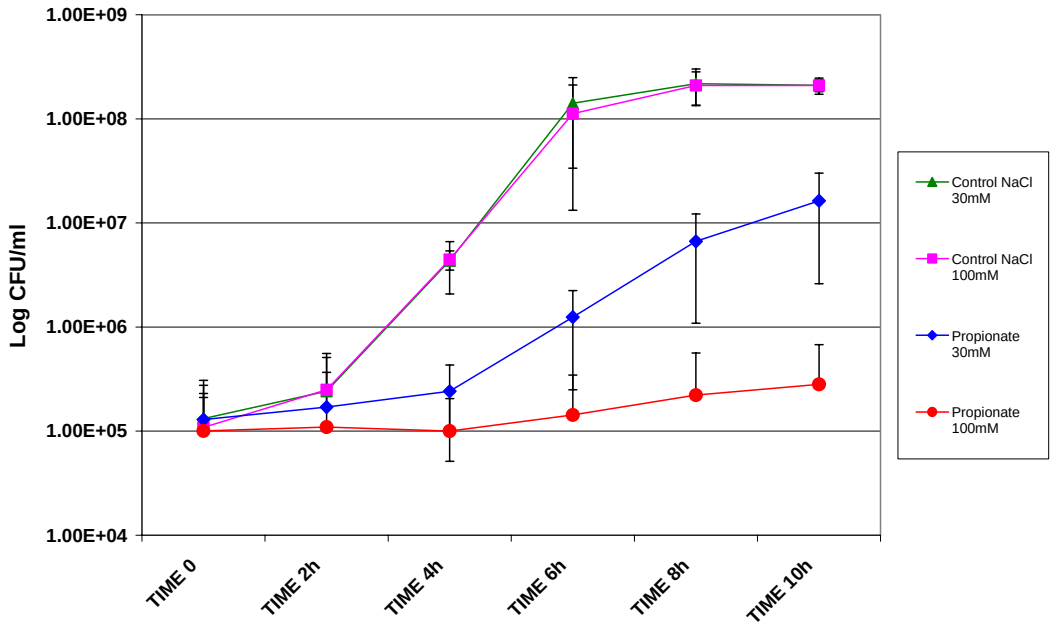

Figure 3-13. The effect of LB broth with sodium propionate at 30mM or 100mM compared to NaCl at 30mM or 100mM on anaerobic growth of *S.* Typhimurium. All solutions were pH 6.5. Error bars represent 95%CI for four replicates of the experiment.

Effect of the Virulence Plasmid and *spv* Genes on Propionate Response

There was no statistically significant effect of the presence of the virulence plasmid with the *spv* genes on the growth of *Salmonella* in the presence of propionate at 30mM or 100mM concentrations. Dissimilar bacterial starting concentrations between the two experiments made the growth curves appear different. Both χ3306 and χ3337 in 100mM sodium propionate remained static over the 10 h experimental period. Extending the

culture times to 18-24 h may have revealed very slow multiplication rates or eventual death of the cells.

In vitro Effects of Cecal Liquor from Antibiotic-treated Horses on Anaerobic Growth of *Salmonella*

Figures 3-14 and 3-15 show the growth curves for *S.* Typhimurium exposed to pooled samples of 10% filter-sterilized cecal contents from five horses treated with the same antibiotic (or control treatment). Growth in LB broth (Figure 3-14) and M9 minimal medium with 20% glucose (Figure 3-15) is evaluated. Whether the medium was nutritionally rich or limiting had no effect, and there was no significant difference between pooled additives for any treatment for either medium.

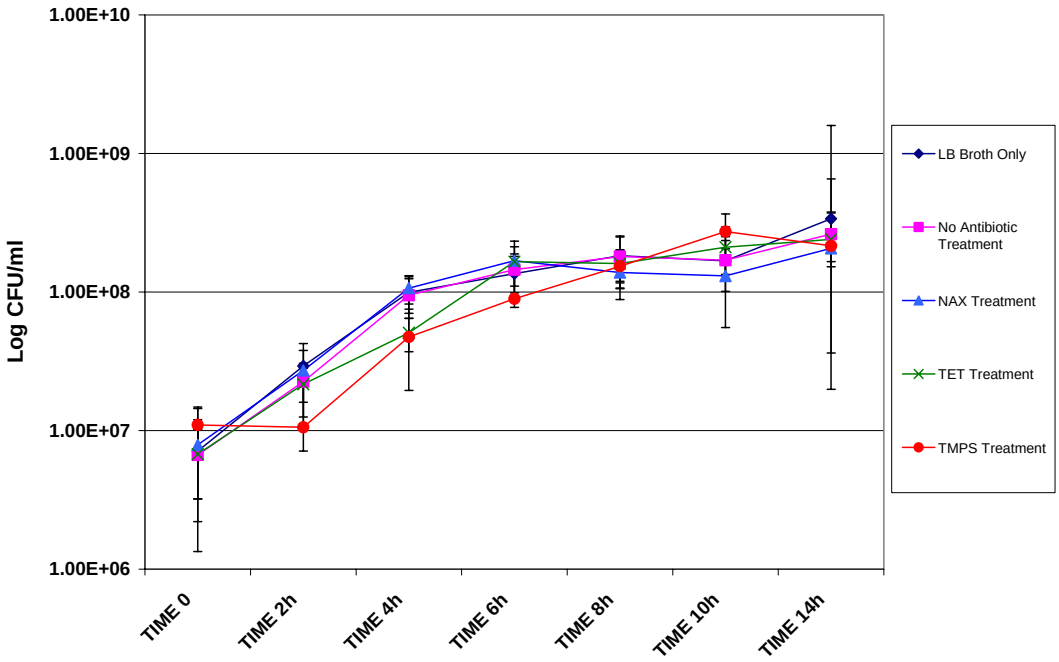

Figure 3-14. The effect of LB broth with 10% added filter-sterilized cecal contents pooled from five horses by treatment on the anaerobic growth of *S.* Typhimurium. The horses were treated with control (no treatment), ceftiofur (NAX), oxytetracycline (TET), or trimethoprim-sulfamethoxazole (TMPS).

Due to inadequate dilution of the starting bacterial inoculum (10^6-10^7 CFU/ml), the growth curves in Figures 3-14 through 3-16 demonstrate only a small portion of the

logarithmic phase of bacterial growth. Differences in growth rate would be most apparent during this phase. The issue was corrected by increasing the dilution of the initial inoculum to lower than 10^5 CFU/ml in further experiments. For all further experiments examining the individual horses, between horse differences were not observed, and means of the five horses were compared between treatments. There were no apparent inhibitory effects of the pooled cecal fluid additions in LB broth (Figure 3-14); however, some treatment additives subjectively appeared to stimulate growth rates in M9 relative to the control (Figure 3-15).

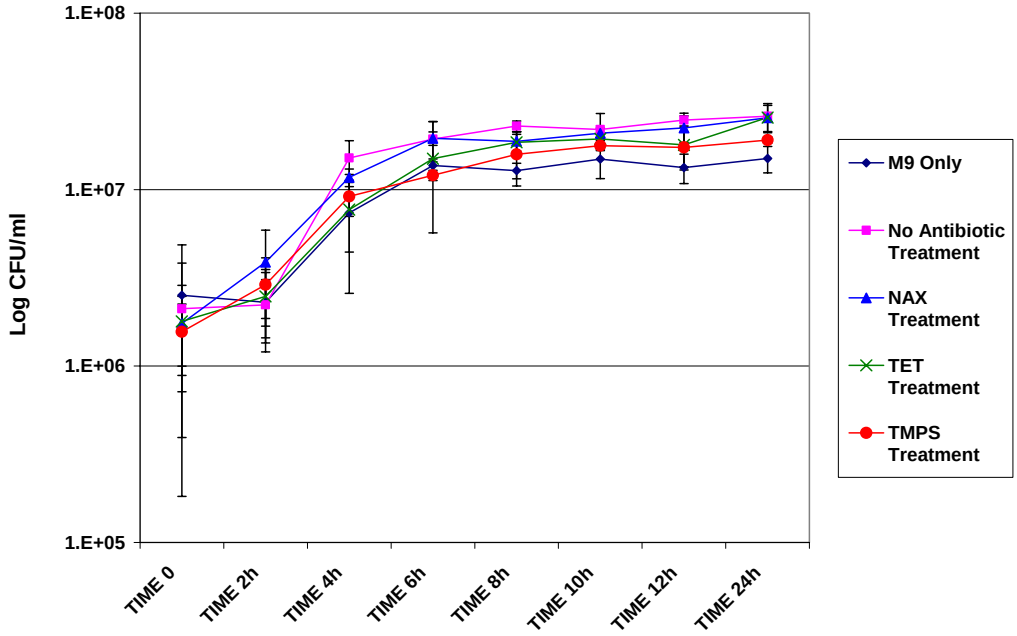

Figure 3-15. The effect of M9 minimal medium (+ glucose) with 10% added filter-sterilized cecal contents pooled from five horses by treatment on the anaerobic growth of *S.* Typhimurium. The horses were treated with control (no treatment), ceftiofur (NAX), oxytetracycline (TET), or trimethoprim-sulfamethoxazole (TMPS).

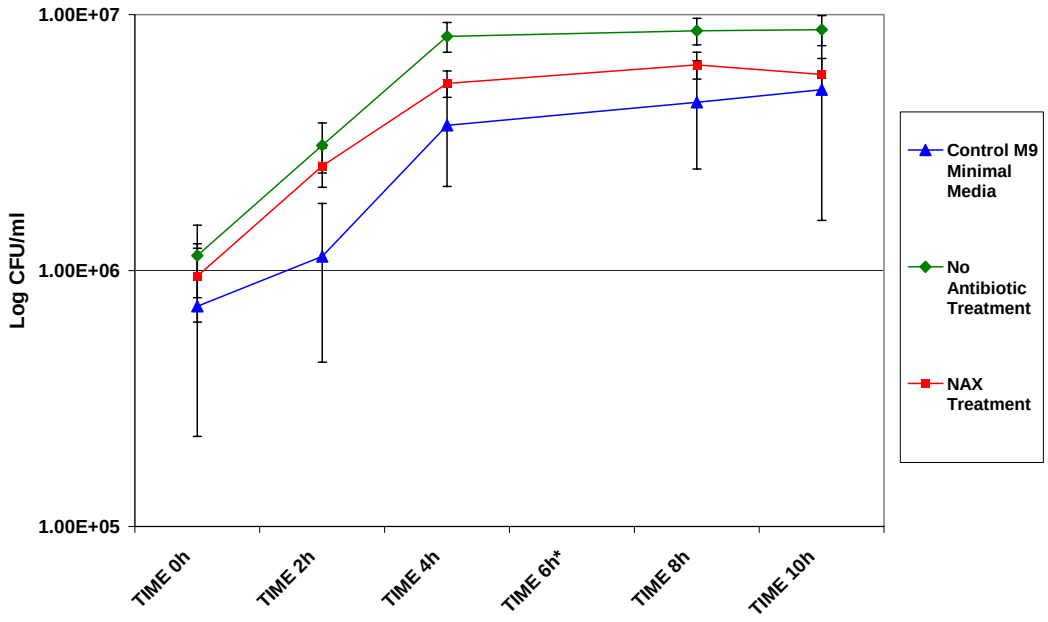

Figure 3-16. The effect of M9 minimal medium (+ glucose) with 10% added filter-sterilized cecal contents from antibiotic-treated horses on the anaerobic growth of *S.* Typhimurium. Data points are the mean of 5 individual horses treated with control (no treatment) or ceftiofur (NAX). Time 6h is a missing data point.

When filter-sterilized cecal contents from horses treated with no treatment (control), ceftiofur sodium (NAX), and trimethoprim-sulfamethoxazole (TMPS) were added in a 10% concentration to anaerobic salmonella cultures in M9 minimal medium (Figures 3-16 and 3-17), they demonstrated increased growth rates relative to M9 alone or M9 with 10% TET treated horse cecal fluid (p=0.001 to 0.005). The stationary phase cell densities appeared similar between all groups, including the controls. There was also no difference between the M9 only control tubes versus the TET treated additive tubes, where the other 3 antibiotic treatments increased growth rates during the logarithmic phase.

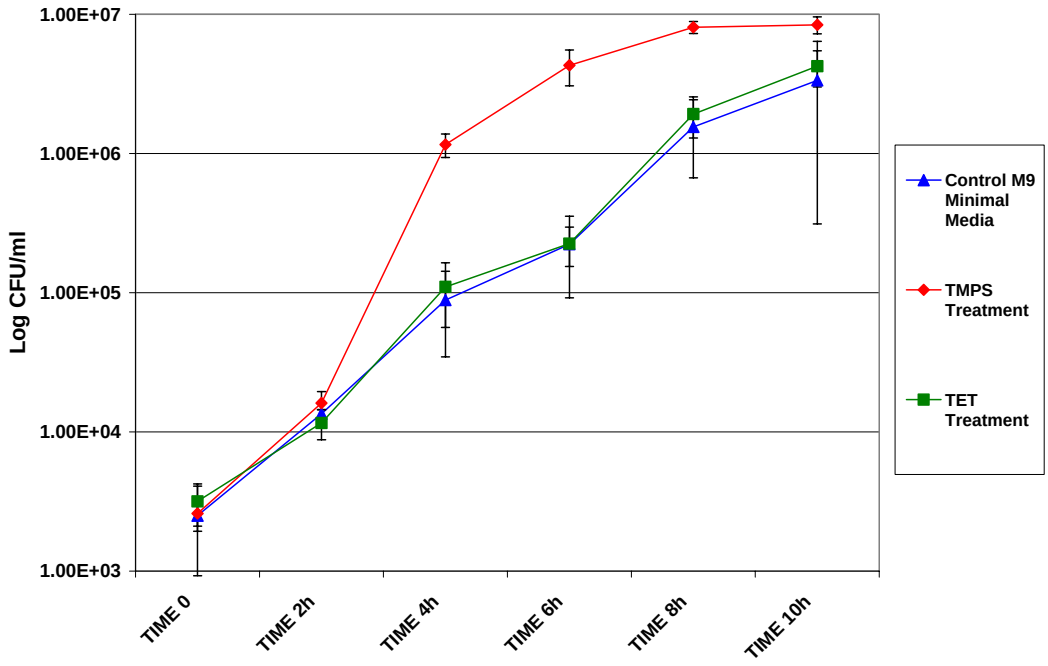

Figure 3-17. The effect of M9 minimal medium (+ glucose) with 10% added filter-sterilized cecal contents from antibiotic-treated horses on the anaerobic growth of *S.* Typhimurium. Data points are the mean of 5 individual horses treated with oxytetracycline (TET) or trimethoprim-sulfamethoxazole (TMPS).

Effect of the Virulence Plasmid and *spv* Genes on Anaerobic Growth of *Salmonella* Exposed to Cecal Liquor from Antibiotic-Treated Horses

Once again, no significant difference could be detected between strains χ3306 and χ3337 in any of the experiments (data not shown), so data from each trial were combined for the two strains and considered replicates. The absence of variation between these strains is not surprising, as no intestinal growth function has been attributed to the virulence plasmid or *spv* genes in any host species.

Discussion

Antibiotic administration had minimal effects on most of the dependent variables measured in this study. One obvious area of contention is the duration of antibiotic treatment given to these experimental animals. In hindsight the four-day duration of

therapy may have been too short to alter the intestinal microenvironment to any statistically significant degree. The selection of four days of treatment was based on anecdotal and experimental data indicating that enteric complications associated with antibiotic usage in horses commonly occur within this period. In clinical equine practice it is not uncommon to treat horses with antibiotics for variable periods, ranging upwards from three days. Antibiotic-associated diarrhea frequently occurs within the initial three days of therapy and there are reports of horses developing severe and fatal diarrhea after a single dose of antibiotic.[205] Several studies examining the effect of antibiotic treatment on development of diarrhea in horses[206] and humans,[207] or on fecal bacterial counts in horses[49] and humans,[85] have concluded that the development of diarrhea, trough bacterial and SCFA levels[82] and minimums in microflora-associated characteristics[86] were usually achieved within three days of commencing oral or parenteral therapy. This supported the selection of a four-day treatment course as any changes in fecal bacterial or SCFA measurements, or biochemical derangements, would likely be reflected by the cecal microenvironment.

Carryover effects of antibiotic treatment on cecal microflora were minimized using a randomized block experimental design with a relatively long inter-treatment period (>30 days). The cecal flora and its biochemical environment are in a constant state of flux, adapting in response to host, diet, season, and other factors. It is logical to assume that antibiotic treatment of the host is a significant event in shaping that adaptive response. Whether or not the microenvironment can actually reconstitute to a normal or a pre-treatment state after an event such as antibiotic therapy was not determined in this

study. Given the minimal treatment effects in the present study it was unlikely that significant carryover effects occurred.

None of the horses in the study developed overt complications of antibiotic treatment, including the development of diarrhea. There was also no apparent reduction in appetite during the treatment period. It has been reported that antibiotic therapy, especially oral therapy, may cause partial or complete anorexia in horses,[61] although this is not a reported complication of any of the antibiotics used in this study. It has been suggested that the risk of antibiotic-associated diarrhea may be potentiated by a reduction in feed intake.[208] The effect of feed intake on antibiotic-mediated changes in intestinal flora could be a worthwhile focus of future studies.

In the present study it was possible that transient diarrhea could have been passed unobserved, but there was no residual evidence of this (wet or soiled tail, perineum, or hocks). Several investigators have reported antibiotic-mediated biochemical changes in fecal and cecal contents in the absence of diarrhea in several species.[49;82-85] Consequently, the absence of diarrhea should not by itself provide an explanation for the minimal observed changes in cecal microflora.

The anticipated effect of antibiotics with activity against anaerobic bacteria would have been an increase in pH. This result should be coincident with a significant reduction in production of SCFAs. Neither effect was observed under the present experimental conditions, with the exception of some changes in individual SCFA concentrations. The time of concentrate feeding and cecal sampling was consistent each day in order to minimize normal temporal and dietary influences on the cecal pH. Under normal conditions it is likely that cecal pH varies, albeit within a relatively small range.

Continuous pH monitoring or more frequent sampling might have yielded different data, but this was considered unlikely. Furthermore, techniques used to continuously or repeatedly record luminal pH are likely to lead to contamination of the anaerobic environment with air.

The significance of luminal pH may not be as important as previously thought, especially in the case of attaching and invasive pathogens such as *Salmonella* spp. It was recently demonstrated that the absorption of SCFAs from the intestinal lumen was relatively independent of the luminal pH.[209] Local pH dictates the ionization status of these acids, and therefore their ability to cross cell membranes. However, the pH at the apical cell surface is near neutral, and remains so in spite of major changes in lumen pH. The regulation of this pH layer is exquisitely dependent on bicarbonate secretion and may explain how invasive pathogens can biochemically recognize a change in location and use that as a signal to attach or invade.

Antibiotic therapy has the potential to disrupt non-bacterial members of the autochthonous flora, namely the protozoa. The most commonly studied ecosystem with respect to autochthonous protozoa is the ruminant forestomach. The species, numbers, and functions of protozoa residing within the cecum and large colon of horses are significantly different than that of ruminants.[154;164;210] Along with bacteria, protozoa are also involved in the terminal digestion and fermentation of polysaccharide foodstuffs, as they function to ferment polysaccharides (minimal contribution to the total), store starches—thus protecting them from bacterial fermentation, as well as regulating the number of resident fungi and bacteria through predatory actions.[211] Indirect effects of

antibiotic therapy could also regulate total numbers of commensal protozoa, through changes in local pH, or metabolites such as SCFAs.

Adam reported wide temporal variations in the cecal protozoa of horses even within an individual on a consistent diet composition[201] and this was corroborated by Moore and Dehority.[210] Reported values for cecal protozoa in the normal, non-fistulated horse range from 4 x 10^3 protozoa per ml contents [154] to 55 x 10^3 per ml.[210] This intra- and inter-animal variability in protozoal numbers was also noted in the present study and made recognition of potential treatment effects difficult. Several investigators have found divergent values for the same characteristic being studied, depending on the whether the animal had ceco-cutaneous access, or was intact and sacrificed.[166] Diet type has also been reported to alter the relative proportions of protozoal species in the cecum of the equine, but not overall numbers.[164] This is in contrast to the equine large intestine, where commensal protozoal numbers were typically 2-3 times greater than in the cecum and their numbers changed significantly in response to increasing the amount of fermentable carbohydrate in the diet.[210]

The variability in protozoal numbers in the present study, coupled with the relatively low number of experimental animals, made investigation of treatment effects difficult. The effect of antibiotic therapy on equine cecal protozoal numbers has not been previously reported, so no assumptions were made prior to experimentation. When examining data from ruminants, it might be hypothesized that antibiotic therapy with an agent encompassing a protozoal spectrum such as metronidazole, the sulphonamides, trimethoprim, the lincosamides,[153] or aminoglycosides[212] would decrease relative numbers of commensal protozoa or change the demographics of their populations.

However, this may be irrelevant in horses, as Moore and Dehority concluded that the commensal protozoa in horses contribute insignificantly to total functional hindgut fermentation.[210]

Significant antibiotic-induced changes in SCFA concentrations were seen in the present study, but these changes were not as great as anticipated based on similar studies in human beings. Previous data in horses unfortunately are restricted to studies involving a single antibiotic treatment making direct comparisons difficult.[170;174] There are several technical reasons that could explain the minimal antibiotic-induced changes. These include a relatively insensitive measuring technique, deterioration of the volatile acids during sample processing and storage, or inappropriate model application (i.e., cannulated animals vs. intact). Measurement of SCFA concentrations at a single point in time does not directly reflect drug-induced changes in production as fluctuations in cecal volume, as well as local SCFA absorption or conversion could distort significant changes associated with treatment. Post-prandial volume of the equine cecum or large colon has been shown to increase from one to four times compared to the pre-fed state,[213] so dilution must be considered when trying to determine production rates or total cecal SCFAs. A constant time between feeding and sampling, coupled with a constant diet in the present study should have minimized point-to-point fluctuations in volume, independent of treatment. Short-chain fatty acid relative proportions to each other as a percentage of the total (molar ratios) may be a better indicator, as cecal volume can only be estimated in the living animal. Unfortunately molar ratios were also not significantly different from controls in antibiotic treated animals.

Oxytetracycline administration had the greatest impact on SCFA concentrations. The association between the tetracycline family of antibiotics and diarrhea in horses has received enormous attention in the veterinary literature. The incidence of diarrhea appears to be greatest when the drug is administered orally.[55] Recently it has been suggested that the incidence of diarrhea after intravenous administration of oxytetracycline has been over-stated (personal communication, G.D. Lester, Murdoch University, Western Australia). Biliary excretion of oxytetracycline after parenteral administration is considered to be minimal.[170] This route of excretion could however become important when high doses of the drug are used. Interestingly, early reports of the association between tetracycline and diarrhea were based on experimental scenarios involving very high parenteral doses of the drug.

The fact that ceftiofur and trimethoprim-sulfamethoxazole had minimal significant impact on total and individual SCFA levels leaves open the pathophysiology regarding diarrhea caused by these compounds. There is recent evidence, albeit anecdotal, that the diarrhea reportedly associated with ceftiofur may be mediated by *Clostridium difficile* (personal communication, G.D. Lester, Murdoch University, Western Australia). The relationship between trimethoprim-sulfamethoxazole preparations and diarrhea may be influenced by alterations in feed intake.[208] Continued investigation into the area of antibiotic effects on the gastrointestinal microenvironment could include more sensitive identification and quantification of naturally occurring and locally active compounds such as SCFAs, bacterial by-products of metabolism, bacterial breakdown compounds such as endotoxin, as well as fluxes in the resident commensal populations of organisms such as yeasts, bacteria, and protozoa.

There was no significant effect of antibiotic treatment on cecal anaerobic bacterial numbers. There was however trends in the data to suggest that trimethoprim-sulfamethoxazole may have reduced bacterial numbers. The medium used to culture and quantitatively examine the flora in biologic anaerobic samples is typically nutritionally rich and non-selective in order to maximize recovery of the more fastidious organisms. A selective antibiotic (gentamicin) was included in the anaerobic culture media in order to reflect a true obligate anaerobic bacterial count. Anaerobic bacteria, due to their decreased trans-membrane electrical potential, are intrinsically resistant to the aminoglycosides, as the drug cannot be transported into the cell[212] (personal communication, M. Cox, Anaerobe Systems, San Jose, CA). This happens to a minor degree in facultative anaerobes undergoing anaerobic respiration. Facultative anaerobes may contribute somewhat to carbohydrate fermentation in the normal animal, though they may play a larger part in hyper-fermentation disorders, such as carbohydrate overload in horses and rumen acidosis in cattle. Facultative anaerobes are also a small minority of the cecal microflora in the horse, as one investigator reported that the overwhelming majority of bacteria in the cecum of the horse were strict anaerobes (> 80%).[154] Few historical quantitative reports on the total anaerobic flora of the rumen or cecum have addressed this issue of bacterial sub-populations, and most used non-selective media under completely anaerobic conditions, which does not differentiate between species of varying oxygen tolerance. de Fombelle reported that large intestinal (cecum and right ventral colon) anaerobic bacteria in cannulated ponies were present at approximately 10^8 CFU/ml, while the aero-anaerobic population was a little more than 10^7 CFU/ml. Since the aero-tolerant anaerobes would be culture-included in the population of the strict

anaerobes, according to this data, they should number approximately 9 x 10^7 CFU/ml.[169] Reported anaerobic bacterial strains isolated from the cecum of *Equidae* include *Butyrivibrios*, *Streptococcus*, *Bacteroides*, *Lactobacillus*, *Selenomonas*, *Eubacteria*, *Proprionibacteria*, *Staphylococcus*, *Veillonella*, *Clostridium*, and *Bifidobacter*.[51;166;171] Oxygen tolerant strains of streptococci, lactobacilli, and staphylococci have been isolated from the horse cecum.[163] In 1973, Kern et al. reported that a larger proportion of the bacteria isolated from the cecum of ponies were facultative anaerobes (as compared to the rumen of steers). The authors hypothesized that either less active fermentation occurred in the pony cecum or that the availability of oxygen was increased.[164] If the latter is indeed true, it could be anticipated that fistulated or cannulated animals will have even lower average counts of anaerobes and greater than average counts of aerotolerant species, depending on the integrity of the cannula.

Discrepancies between direct smear counts and viable or culturable organisms have been reported in two studies.[163;164] This is likely related to the fastidiousness or oxygen tolerance of the organisms, and was an equally important concern in this work. The fact that a partially selective medium was used in conjunction with chronically cannulated animals, can easily explain why the actual numbers of culturable anaerobes in this study were consistently lower than other reported values for the equine cecum. Also, differences in measurement and reporting units (e.g., CFU per volume of contents vs. weight of contents) further complicate comparison between studies. Furthermore, our experimental collection technique does not address an important population of bacteria that are adherent to the mucosa.[214] This adherent population of bacteria was included in a study of equine cecum, but the authors made no distinction between luminal bacterial

numbers and adherent numbers in their results.[166] Ideally, studies where the animals were sacrificed and the gastrointestinal tract was immediately opened, and contents as well as mucosal scrapings were collected may give a more accurate representation of the true numbers of bacteria.

Our data failed to demonstrate marked effects of antibiotics on SCFA concentrations *in vivo*. Consequently, it is difficult to directly implicate changes in SCFA concentrations in the pathogenesis of antibiotic-associated diarrhea. Nevertheless, manipulation of luminal SCFA concentrations may still be an important focus of treatment and prevention of salmonella infection, particularly in high-risk patients. It is apparent from our data that acetate, propionate and butyrate inhibit anaerobic growth of *Salmonella* in a concentration-dependent manner. Propionate and butyrate at 100mM concentrations were most inhibitory, followed by propionate at 30mM and acetate at 100mM. Further investigation with more appropriate physiologic concentrations is now warranted. Once an effective breakpoint is determined; methods of manipulating the endogenous ecosystem to approximate those values become an important next step. Also, this study only looked at single SCFAs, it is quite probable that combination cocktails may yield more significant results.

We initially hypothesized that certain SCFAs would be inhibitory to growth of the *Salmonella* and that antibiotics would, by reducing anaerobic bacterial numbers, be associated with reduced concentrations of SCFAs. It was anticipated that if this hypothesis were true that addition of pathogenic bacteria to sterile cecal liquor from antibiotic-treated horses would demonstrate enhanced growth when compared to cecal liquor collected from horses that had not been treated. Unfortunately, data reported

above confirmed that the effect of antibiotics on cecal contents was minimal. Given this the finding that *Salmonella* grew equally well in the untreated control, trimethoprim-sulfamethoxazole treated, and ceftiofur treated cecal liquor was not unexpected. These data were consistent between plasmid/*spv* bearing and plasmid/*spv* deficient strains of *Salmonella*. What was not expected was the finding that salmonella growth appeared to be slowed when added to the cecal liquor of horses treated with oxytetracycline. There may have been residual active drug in the samples, although Horspool determined that less than 17% of the amount of oxytetracycline administered intravenously reaches the cecal liquor.[170] It is also possible that changes in other organic acids which were not measured, such as lactate, could also have influenced bacterial growth rates.

Another interesting finding was that the growth of *Salmonella* in all cecal liquor supplemented media, with the exception of that collected from oxytetracycline-treated horses, was greater than that in the M9 medium, which is nutritionally complete for *Salmonella* spp. This indicated that sterile cecal fluid (even after antibiotic treatment) has intrinsic nutritive value relative to the minimal medium alone.

CHAPTER 4
SUMMARY, CONCLUSIONS, AND FUTURE DIRECTIONS

There is a dearth of information regarding the molecular characteristics of salmonellae associated with infection of horses. The initial aim of the study was to contrast two populations of horses with respect to molecular characteristics, specifically the presence of bacterial plasmids and *spv* genes. The populations investigated included a symptomatic hospitalized group at the Veterinary Medical Teaching Hospital at the University of Florida and a population of asymptomatic animals from horse properties in North Central Florida. We initially hypothesized that salmonellae recovered from asymptomatic horses would be less likely to contain virulence genes than those associated with clinical disease. Unfortunately, no salmonellae were isolated from asymptomatic horses. We considered several possibilities why this occurred, including the use of a relatively insensitive method of detection, the problem of intermittent shedding, and the interference of additional fecal compounds on bacterial growth. The technique used in the present study of enrichment and culture remains the gold standard of diagnosis, although more sensitive methods such as PCR are available. Our need for live bacteria for additional molecular and sensitivity testing made utilization of PCR impractical for our purposes.[106] Our ability to recover the bacteria from fecal samples was validated on numerous occasions through positive culture of hospitalized animals. The most likely explanation for the failure to recover salmonellae from asymptomatic animals is that horses in this region have an extremely low carriage rate of salmonellae.

Given the wide variation in reported rates of bacterial shedding this observation should not be considered novel.

The focus of the study shifted to a primarily descriptive assessment of the molecular characteristics of bacteria recovered from symptomatic animals. In addition we examined some clinical and laboratory factors that were associated with systemic spread and mortality.

The demographics of affected animals appeared to reflect that of the entire hospital although this comparison was not investigated statistically due to a difficulty in obtaining accurate data of the hospital population for this period. The diseased population was comprised of large number of juvenile animals; interestingly the case outcome was worse in horses less the 4 years of age. A likely explanation was that neonatal foals, when affected with *Salmonella* frequently became bacteremic with secondary sites of infection.

More than 70% of the isolates were obtained from fecal samples, which is consistent with the entero-environmental life cycle of these bacteria.[215] Multiple serovars were isolated from six animals, which demonstrate the fact that different serovars of these organisms can simultaneously co-infect a single host.

Carriage of virulence plasmids, as defined by the presence of *spv* genes, was restricted to certain Group B serovars. These isolates included the serovars Typhimurium, Typhimurium var. Copenhagen, and 4,5,12:i-monophasic, an antigenically close relation to *S.* Typhimurium. The virulence plasmid and *spv* genes have been shown to be important in the calf model of salmonella pathogenesis.[10] Isolates recovered systemically were more likely to be *spv*-gene positive. It was also not surprising that the presence of virulence plasmids also conferred a higher case mortality. These findings are

consistent with data collected in other species. Our data set was not sufficiently robust to allow us to retrospectively examine the relationship between *spv* genes and severity of enteric disease. A prospective clinical study examining the disease severity with respect to these genes would be required to investigate this association.

More than 64% of the clinical isolates examined carried a large (> 20-kb) plasmid, but only 20% of these isolates carried a virulence plasmid. Speculation as to what the other plasmids could potentially be led to the preliminary investigation of antibiotic resistance determinants.

Three isolates were selected on the basis of appropriate antibiotic sensitivities and yielded transference of ceftiofur, cefazolin, and ampicillin resistance. Likely the majority of these large plasmids contain R determinants, but further examinations would be required in order to increase the accuracy of this statement. Resistance to common antimicrobial agents is emerging issue of concern regarding *Salmonella* in human medicine. Monitoring of resistance levels in endemic strains of pathogens such as *Salmonella* allows clinicians to remain aware of the selective pressures placed on these organisms. Four serovars of *Salmonella* accounted for the 22 multidrug-resistant isolates identified in this study (resistant to ≥ 8 drugs out of the 12 clinically relevant drugs tested). *S.* Java (10), *S.* Typhimurium var. Copenhagen (5), *S.* Javiana (2), and *S.* Newport (1). This may be more a reflection of the population being studied (sick, surgical, and hospitalized patients) as they are the population most likely to be receiving these drugs.

The second major phase of the study was to investigate the relationship between antimicrobial therapy and salmonella growth. We applied a combination of *in vivo* and *in*

vitro techniques to investigate this, as we wanted to avoid animal inoculation with *Salmonella*. The initial stage involved examining the effect of antibiotics on cecal microflora using Thoroughbred horses with cecal cannulae. Dependent variables examined included cecal anaerobe counts, cecal pH, and cecal SCFA concentrations.

There were no significant differences detected in total anaerobe counts with respect to antibiotic treatment, but several significant changes in SCFA concentrations were observed. A relationship between these variables is assumed, but our methods and experimental numbers were likely insufficient to detect significant differences in bacterial counts. Newer techniques involving quantitative PCR with species-specific primers are much more sensitive as they do not require the organisms to be viable.[216;217]

Antibiotics were shown to have a significant effect on several SCFA concentrations, but in general the magnitudes of the changes observed were mild. Changes in the control group raised some concerns regarding the preparation used in the study. Likely our data would have been strengthened by a combination of increased numbers of experimental animals and/or repeated sampling of contents to reduce variability. There are problems associated with single point sampling, most importantly is the impact of the unmeasured variable intestinal volume on concentration. Effort was made in the experimental design to minimize any impact of diet type and timing relative to collection, but changes in intestinal volume induced by antibiotic treatment would not have been detected.

Oxytetracycline had the greatest impact on SCFA concentration. The relationship between the tetracycline family of antibiotics and diarrhea is controversial, but the literature indicates that diarrhea can occur in response to treatment.[52;53;55] Our data

indicate that there is an effect of oxytetracycline on cecal flora, albeit of small magnitude. This is most likely due to the limited biliary excretion of the drug.

We then determined that SCFA were inhibitory to equine isolates of *Salmonella* using *in vitro* techniques. There was a concentration-dependant inhibition of anaerobic salmonella growth by SCFA solutions. This was independent of *spv* gene presence and further supports the hypothesis that the *spv* genes are not involved in the enteric phase of the disease in horses. Propionate and butyrate at 100mM concentrations were most inhibitory, followed by propionate at 30mM and acetate at 100mM. These concentrations are considered supraphysiologic, but demonstration of *in vitro* inhibition provides the basis for additional *in vivo* investigations. The focus of additional studies would likely be to identify supplements that could be utilized not only therapeutically, but also prophylactically during periods of increased pathogen susceptibility. SCFA-treated starches fed to rats increased the colonic levels of those acids.[193] Treated feedstuffs or supplements would be readily accepted by horse owners and veterinarians interested in minimizing the side-effects of antimicrobial therapy.

Based on the data collected during the first phase of the antibiotic study it was unlikely that we would be able to demonstrate significant enhancement or inhibition of growth with the liquor from antibiotic treated horses. Indeed, there were no differences between treatments for ceftiofur, trimethoprim-sulfamethoxazole, and control (no treatment) on the anaerobic growth rates of *Salmonella* when they were exposed to 10% solutions of sterilized cecal liquor from antibiotic-treated horses. There were however two interesting, if not unexpected findings. The cecal liquor collected from oxytetracycline-treated animals appeared to inhibit *in vitro* salmonella growth relative to

that of control, ceftiofur, or trimethoprim-sulfamethoxazole treated cecal liquor. One likely explanation may be that residual antibiotic may have suppressed growth in this treatment group. Of additional interest was that control cecal liquor enhanced growth over glucose-supplemented media indicating that growth of bacteria is facilitated by factors within the cecal liquor.

Based on these results, future work investigating the indirect impact of therapeutic antimicrobial therapies on commensal gastrointestinal flora in humans and horses should be encouraged. Antibiotic-associated diarrheas, especially those attributable to *Salmonella* spp. are important causes of morbidity and mortality worldwide, and efforts to understand the pathophysiology behind antibiotic-host-pathogen interactions may lead to novel and economic preventative modalities or therapeutics.

APPENDIX A
SALMONELLA EPIDEMIOLOGY DATA COLLECTION SHEET

Page 1: Salmonella epidemiology survey

Salmonella Epidemiology Data Collection Sheet **admission date:**_____

MR Number..... _____

Age (at admission) ..... _____

Sex..... Mare Stallion Geld

Breed ..... TB QH Stbd Arab

_____

Presenting Complaint..... _____

Surgery / Anesthesia. ..... Yes No date: _____

LIVED.....DIED.....EUTH

Total Bill ..... $_____

Ab tx w/in 1mo of adm..... Yes No Undet.

Ab and dose rate..... _____

Ab #2 and dose rate ..... _____

Ab tx during hospitalization..... Yes No

Ab #1 and dose rate ..... _____

Ab #2 and dose rate ..... _____

Ab #3 and dose rate ..... _____

Duration of hospitalization ..... _____

Diarrhea ..... Yes No

When?..... _____

Duration of diarrhea..... _____days

Number of + fecal cultures..... _____

Page 2: Salmonella epidemiology survey

CBC (at time of + culture)..... leukocytes _____ date:_____
..... neutrophils _____
..... toxicity _____
..... fibrinogen _____
..... platelets _____

Ab resistance data:
..... Amikacin R S _____
..... Ampicillin R S _____
..... Ceftiofur R S _____
..... Enrofloxacin R S _____
..... Gentamicin R S _____
..... TMP-Sx R S _____

Isolate site..... _____
Plasmid ..... Yes No
spv locus ..... Yes No
Salmonella group ..... A B C1 C2 D E
Salmonella serotype..... _____
Salmonella #2 group ..... A B C1 C2 D E
Salmonella #2 serotype..... _____

Location in hospital..... stall _____
Location #2 in hospital..... stall _____

TP at admission..... _____
TP at death or d/c..... _____

APPENDIX B
INDEX OF SUPPLIERS AND CONTACT INFORMATION

Corning Incorporated Life Sciences 45 Nagog Park Acton, MA 01720 Tel: 978-635-2200 Tel: 800-492-1110 Fax: 978-635-2476 http://www.corning.com/lifesciences/US-Canada/en/	Bio-Rad Laboratories 1000 Alfred Nobel Drive Hercules, CA 94547 Tel: 510-724-7000 Fax: 510-741-5817 http://www.biorad.com
JA Webster Veterinary Supply 86 Leominster Road Sterling, MA 01564-2198 Tel: 800-225-7911 Tel: 978-422-8211 Fax: 978-422-8959 http://www.jawebster.com/index.html	Supelco Chromatography Tel 800-247-6628 http://www.sigmaaldrich.com/Brands/Supelco_Home.html
MJ Research, Inc. 5350 Capital Court, #102 Reno, NV 89502 Tel: 888-652-9253 (888-MJCYCLE) 888-735-8437 (888-PELTIER) http://www.mjresearch.com	Sheldon Manufacturing 300 N. 26th Avenue Cornelius, OR 97113 Tel: 800-322-4897 503-640-3000 http://www.shellab.com
Anaerobe Systems 15906 Concord Circle Morgan Hill, CA 95037 Tel: (408) 782-7557 Fax: (408) 782-3031 www.anaerobesystems.com	Sigma-Aldrich Chemical Corp. 3050 Spruce Street St. Louis, MO 63103 Tel: 800-521-8956 http://www.sigmaaldrich.com
bioMérieux, Inc. 100 Rodolphe Street Durham, NC 27712 Tel: (919) 620 20 00 Fax: (919) 620 22 11 http://www.biomerieux-usa.com	Fisher Scientific International Inc. Hampton, NH 03842 Tel: (603) 926-5911 Fax: (603) 929-2379 www.fisherscientific.com

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Microsoft Corporation One Microsoft Way Redmond, WA 98052-6399 http://www.microsoft.com	Perkin Elmer Corporation Life and Analytical Sciences Division 549 Albany Street Boston, MA 02118, USA Tel: (617) 482-9595 Customer Care: 800-762-4000 (USA) http://www.perkinelmer.com
Invitrogen Corporation (formerly Gibco BRL) 1600 Faraday Avenue Carlsbad, California 92008 Tel: (760) 603-7200 Fax: (760) 602-6500 http://www.gibcobrl.com	Qiagen Inc. 28159 Avenue Stanford Valencia, CA 91355 Tel: 800-426-8157 Fax: 800-718-2056 http://www.qiagen.com
UVP Incorporated BioImaging Systems Group 2066 W. 11th Street Upland, CA 91786 Tel: 800-452-6788 or 909-946-3197 Fax: 909-946-3597 http://www.uvp.com	TREK Diagnostic Systems, Inc. 982 Keynote Circle, Suite 6 Cleveland, Ohio 44131 USA Tel: 216-351-TREK (8735) Toll Free: 800-871-8909 Fax: 216-351-5456 Technical Support: 1.800.642.7029 www.trekds.com

APPENDIX C
SALMONELLA ISOLATE INDEX

Table C-1. Index of *Salmonella* isolates by group and serovar, with plasmid and *spv* gene status

Isolate Identification Number (Case)	**Plasmid**	***spv***	**Group**	**Serovar**
1	–	–		Unknown
2	+	+		Unknown
3	+	+	B	Typhimurium
4	+	–	E	Anatum
5	+	–	C_2	Newport
6	+	+	B	Typhimurium
7	+	+	B	Typhimurium
8	+	–	B	Typhimurium
9	+	–	B	Java
10	+	+	B	4,5,12:i-monophasic
11	+	+	B	4,5,12:i-monophasic
12	+	–	C_2	Newport
13	+	–	B	Java
14	+	–	C_2	Newport
15	+	–	B	Java
16	+	–	B	Java
17	+	–	B	Java
18	–	–	B	Agona
19	+	–	B	Typhimurium var. Copenhagen
20	+	–	B	Java
21	+	+	B	4,5,12:i-monophasic
22	–	–	B	Typhimurium var. Copenhagen
23	+	+	B	Typhimurium
24	+	–	C_1	Hartford
25	+	+	B	Typhimurium
26	+	+	B	Typhimurium var. Copenhagen
27	+	–	B	Java
28	+	–	B	Java
29	+	–	B	Typhimurium var. Copenhagen
30	–	–	B	Java
31	+	–	D	Javiana

Table C-1. Continued

Isolate Identification Number (Case)	Plasmid	*spv*	Group	Serovar
32	–	–	C_1	Braenderup
33	+	–	B	Java
34	+	–	C_2	Newport
35	–	–	E	Anatum
36	+	–	F	Rubishlaw
37	+	–	C_2	Muenchen
38	–	–	D	Javiana
39	–	–	C_2	Newport
40	+	–	B	Typhimurium var. Copenhagen
41	+	–	B	Java
42	+	–	---	Unknown
43	+	–	B	Typhimurium var. Copenhagen
44	+	–	B	Typhimurium var. Copenhagen
45	–	–	E	London
46	+	–	C_2	Muenchen
47	+	–	C_2	Newport
48	+	+	B	Typhimurium
49	+	+	B	Typhimurium
50	–	–	B	SaintPaul
51	+	–	C_1	Mbandaka
52	–	–	C_2	Newport
53	–	–	D	Miami
54	–	–	B	SaintPaul
55	+	–	B	Java
56	+	–	C_2	Muenchen
57	+	–	B	Java
58	–	–	D	Miami
59	+	–	B	Java
60	–	–	B	SaintPaul
61	+	–	B	Java
62	–	–	D	Miami
63	+	–	E	London
64	–	–	E	Meleagridis
65	+	–	D	Javiana
66	–	–	C_2	Newport
67	–	–	D	Miami
68	+	–	C_2	Muenchen
69	+	–	D	Javiana

Table C-1. Continued

Isolate Identification Number (Case)	Plasmid	*spv*	Group	Serovar
70	–	–	B	Java
71	–	–	D	Miami
72	+	–	B	SaintPaul
73	+	–	C_1	Hartford
74	+	–	C_2	Muenchen
75	+	–	B	Java
76	–	–	C_1	Infantis
77	–	–	D	Miami
78	+	–	B	Java
79	–	–	D	Javiana
80	+	–	C_2	Muenchen
81	–	–	B	Multiple B Serovars
82	–	–	B	SaintPaul
83	–	–	B	Reading
84	+	–	E	Anatum
85	+	–	B	Java
86	+	–	B	Java
87	–	–	C_2	Newport
88	+	–	B	Java
89	+	+	B	Typhimurium var. Copenhagen
90	–	–	C_2	Tallahassee
91	–	–	C_2	Newport
92	+	–	B	Java
93	+	–	D	Miami
94	–	–	E	Newington
95	–	–	C_2	Newport
96	+	–	B	Java
97	+	–	B	Java
98	+	–	B	Java
99	–	–	C_2	Newport
100	–	–	E	Anatum
101	+	–	B	SaintPaul
102	–	–	C_2	Newport
103	–	–	E	Newington
104	–	–	C_1	Mbandaka

APPENDIX D
SALMONELLA DATABASE CASE DESCRIPTIVE INFORMATION

This appendix contains the complete database of descriptive information obtained regarding each clinical isolate of *Salmonella* examined in this study, displayed in Tables D-1 through D-3. Isolate coding has been assigned to conceal identifying client and patient information. Blank cells indicate that the record was missing or otherwise not available and annotation stating that the information could not be determined from the record and patient history is shown in cases where the record was reviewed.

Table D-1. Salmonella case descriptive information: breed, age, sex, presenting complaint, risk factors for salmonellosis, specimen origin, and salmonella group(s) and serovar(s). Blank cells indicate missing or unavailable records.

Case ID	Breed	Age	Sex	Presenting Complaint	Clinical Syndrome	Risk Factors	Specimen Origin	Salmonella Group	Serovar	2nd Salmonella Group	2nd Serovar
1	QH	14y	M	Febrile, colic	Colic - No Diarrhea	Colic	Feces	C2	unknown	N/A	N/A
2	Unknown	13y	F		Diarrhea		Feces	B	unknown	N/A	N/A
3	TB	9y	F	Diarrhea, weight loss, late in foal	Diarrhea, Weight Loss	Pregnant	Feces	B	typhimurium	N/A	N/A
4	Paso Fino	3y	M	Diarrhea, colic - SC impaction. Ex Lap, did badly post-op, severe endotoxemia, died spontaneously	Colic - Diarrhea	Colic, Anesthesia / Surgery	Feces	E	anatum	N/A	N/A
5	Arab	12y	M	Colic	Diarrhea	Colic	Feces	C2	newport	N/A	N/A
6	Standardbred	2y	F	Diarrhea, fever	Diarrhea, Fever		Feces	B	typhimurium	N/A	N/A
7	TB	2wks	M	Fever, swollen joint, diarrhea	Synovitis, Fever, Diarrhea	Systemic Disease	Joint Fluid	B	typhimurium	N/A	N/A
8	Paso Fino	2y	F	Chronic diarrhea (6 weeks)	Diarrhea - Chronic	None Determined	Feces	B	typhimurium	N/A	N/A
9	Standardbred	2y	F	Colic, fever, reflux, anterior enteritis, previously admitted (6 d. prior) to VMTH for scintigraphy and CSF tap. Exploratory laparotomy showed 3m proximal jejunum thickened w/serosal ecchymoses	Colic - No Diarrhea, Fever	Colic, Anesthesia / Surgery	Duodenum - Necropsy	B	java	N/A	N/A
10	TB	2d	F	Diarrhea, DOA	Diarrhea	Neonate	SI - Necropsy	B	4,5,12 : i- monophasic	N/A	N/A
11	TB	1m	M	Colic, GDUD, S. equi equi abscess, collapsing trachea	Colic - No Diarrhea	Systemic Disease (non-colic), Colic	LI - Necropsy	B	4,5,12 : i- monophasic	N/A	N/A

Table D-1. Continued

Case ID	Breed	Age	Sex	Presenting Complaint	Clinical Syndrome	Risk Factors	Specimen Origin	Salmonella Group	Serovar	2nd Salmonella Group	2nd Serovar
12	Paint	1y	M	Diarrhea, foaming at mouth, coughing since show 1m prior, currently on "cough medicine", given Probios® 1d prior to admission	Diarrhea	Systemic Disease (non-colic)	SI, SC, Lymph Node - Necropsy	C2	newport	N/A	N/A
13	Warmblood	5y	M	Shipped from Belgium to NY, NY to MD, MD to FL in the week prior to presentation. Developed fever and respiratory disease	Diarrhea, Fever	Shipping, Systemic Disease (non-colic)	Feces	B	java	N/A	N/A
14	QH	1mo	M	Diarrhea, bucket-fed orphan foal (mare died 8h post-partum)	Diarrhea	Antibiotic Administration	Feces	C2	newport	N/A	N/A
15	QH	7y	M	Colic - cecal impaction, surgical correction, post-op diarrhea	Post-Op Colic Diarrhea	Colic, Anesthesia / Surgery	Feces	B	java	N/A	N/A
16	TB	5m	F	Pneumonia	Diarrhea, Pneumonia	Antibiotic Administration, Systemic Disease	Feces	B	java	N/A	N/A
17	Mix Breed	20y	M	Diarrhea, post-op surgery for incisor removal, colic episode 4d prior to surgery. Has had repeated leukopenic episodes since desmotomy surgery 2y prior	Post-Op (non-colic) Diarrhea	Anesthesia / Surgery, Colic	Feces	B	java	N/A	N/A
18	Unknown			Necropsy	Necropsy - unknown COD	None Determined	LI - Necropsy	B	agona	N/A	N/A
19	TB	1y	F	Shipped from KY 9d. prior to presentation. Post-op stapling bilateral carpi, developed diarrhea and fever	Post-Op (non-colic) Diarrhea	Shipping, Anesthesia / Surgery	Feces	B	typhimurium (copenhagen)	N/A	N/A

Table D-1. Continued

Case ID	Breed	Age	Sex	Presenting Complaint	Clinical Syndrome	Risk Factors	Specimen Origin	Salmonella Group	Serovar	2nd Salmonella Group	2nd Serovar
20	TB	14y	M	Fever, diarrhea, post-op epiploic foramen entrapment, SI resection	Post-Op Colic Diarrhea, Fever	Colic, Anesthesia / Surgery	Feces	B	java	N/A	N/A
21	TB	1m	M	Diarrhea, fever	Diarrhea, Fever	None Determined	Feces	B	4,5,12 : i- monophasic	N/A	N/A
22	TB	1.5y	M	Fracture distal humerus, anesthesia for radiographs	Diarrhea	Anesthesia, Systemic Disease	Feces	B	typhimurium (copenhagen)	N/A	N/A
23	TB	1y	M	Colic, nephrosplenic entrapment and colon torsion, died of colon rupture 8d post surgery	Post-Op Colic Diarrhea	Anesthesia / Surgery, Colic	Feces	B	typhimurium	N/A	N/A
24	TB	2m	F	Bilateral stifle septic arthritis / osteomyelitis	Synovitis, Osteomyelitis, Diarrhea	Antibiotic Administration, Systemic Disease	Joint Fluid	C1	hartford	N/A	N/A
25	TB	1m	F	Shipped from IN 5d before presentation, pneumonia, polysynovitis, pericarditis, sepsis, fever	Bacteremia, Diarrhea	Shipping	Duodenum - Necropsy	B	typhimurium	N/A	N/A
26	Paso Fino	1.5m	F	Chronic diarrhea. Previously admitted to VMTH 6d earlier for diarrhea (presumed sand induced). *Salmonella* recovered from physis at necropsy (isolate not saved)	Diarrhea - Chronic	Antibiotic Administration	Feces	B	typhimurium (copenhagen)	N/A	N/A
27	TB	3m	M	Dysphagic since guttural pouch fistulation surgery 1month prior. Aspiration pneumonia.	Pneumonia, Diarrhea	Anesthesia / Surgery, Systemic Disease	Feces	B	java	N/A	N/A

Table D-1. Continued

Case ID	Breed	Age	Sex	Presenting Complaint	Clinical Syndrome	Risk Factors	Specimen Origin	Salmonella Group	Serovar	2nd Salmonella Group	2nd Serovar
28	TB	1m	M	Umbilical herniorrhapy, incisional infection, hernia recurrence, repeat herniorrhapy	Post-Op (non-colic) Diarrhea	Anesthesia / Surgery	Feces	B	java	N/A	N/A
29	Arab	2y	M	Splinters discovered in coronary band (puncture wound) - developed colitis and laminitis during hospitalization	Diarrhea, Laminitis	Antibiotic Administration, Systemic Disease (non-colic), Colic	Feces	B	java	N/A	N/A
30	QH	7y	M	Chronic stress colicker per owner, presented for colic / colon torsion / proximal enteritis. Rancid gastric contents pre-surgery and watery SI contents at surgery	Post-Op Colic Diarrhea, Chronic Colic	Colic, Anesthesia / Surgery	Feces	B	unknown - NVSL sample contaminated	N/A	N/A
31	Warmblood	9y	M	Diarrhea, diagnosed *Salmonella* positive 2y previous	Diarrhea	None Determined	Feces	D	javiana	N/A	N/A
32	TB	2y	M	Diarrhea, fever, given Probios® 2d before presentation as therapy	Diarrhea, Fever	Antibiotic Administration	Feces	C1	braenderup	N/A	N/A
33	Mix Breed	14y	F	SI entrapment unidentified	Colic - No Diarrhea	Colic	SI - Necropsy	B	java	N/A	N/A
34	QH	11y	F	Colic - large colon impaction	Colic - Diarrhea	Colic	Feces	C2	newport	N/A	N/A
35	QH	14y	F	Diarrhea, presented to VMTH 7d. prior for large colon displacement, several passages of NG tube and developed diarrhea. Given Probios® day of admission.	Colic - Diarrhea	Colic	Feces	E	anatum	N/A	N/A

Table D-1. Continued

Case ID	Breed	Age	Sex	Presenting Complaint	Clinical Syndrome	Risk Factors	Specimen Origin	Salmonella Group	Serovar	2nd Salmonella Group	2nd Serovar
36	Miniature Horse	7y	M	Fever, diarrhea, anorexia	Diarrhea, Fever	None Determined	Feces	F	rubishlaw	C2	muenchen
37	QH	3m	M	Colic (SI volvulus), chronic diarrhea for 3 mo.	Colic - Diarrhea, Chronic Diarrhea	Anesthesia / Surgery, Colic	Feces	C2	muenchen	B	Multiple serovars (NVSL sample)
38	Arab	20y	F	Chronic colic	Chronic Colic - No Diarrhea	Colic	Feces	D	javiana	N/A	N/A
39	Miniature Horse	6y	F	Diarrhea, fever. "Every summer gets intermittent diarrhea."	Diarrhea, Fever	None Determined	Feces	C2	newport	C1	hartford
40	Paso Fino	2y	F	Diarrhea. Admitted to VMTH 2 weeks prior with mild pleuropneumonia, treated with antibiotics	Diarrhea	Antibiotic Administration, Systemic Disease	Feces	B	typhimurium (copenhagen)	N/A	N/A
41	Draft Breed	6y	M	Colic (ileal impaction), corrective surgery	Post-Op Colic Diarrhea	Colic, Anesthesia / Surgery	Feces	B	java	N/A	N/A
42	QH	4m	M	Presented for B. bronchiseptica pneumonia approx. 2 weeks prior. Re-presented for diarrhea	Diarrhea	Systemic Disease (non-colic)	Feces	B	unknown (NVSL sample contaminated)	N/A	N/A
43	Paso Fino	4m	M	Diarrhea. Diagnosed *Salmonella* positive horse stalled next to mare and foal at farm. Diarrhea started 4d after treatment for respiratory disease initiated.	Diarrhea	Antibiotic Administration, Exposure to known Salmonella + Horse	Feces	B	typhimurium (copenhagen)	N/A	N/A
44	Standardbred	3y	M	Fever, pneumonia. Developed small colon and cecal impaction 2d after presentation.	Colic - Diarrhea, Fever	Colic, Antibiotic Administration, Systemic Disease (non-colic)	Feces	B	typhimurium (copenhagen)	N/A	N/A

Table D-1. Continued

Case ID	Breed	Age	Sex	Presenting Complaint	Clinical Syndrome	Risk Factors	Specimen Origin	Salmonella Group	Serovar	2nd Salmonella Group	2nd Serovar
45	Pony	3y	F	Colic - PF impaction	Colic - Diarrhea	Colic	Feces	E	london	N/A	N/A
46	Unknown	38y					LI - Necropsy	C2	muenchen	N/A	N/A
47	TB	1.5y	M	Colic, diarrhea, fever. History of diarrhea before presentation. Received plasma during treatment	Post-Op Colic Diarrhea, Fever	Anesthesia / Surgery, Colic	Feces	C2	newport	N/A	N/A
48	TB	2m	F	Diarrhea, severe GDUD, perforated duodenum	Diarrhea	Colic	SI - Necropsy	B	typhimurium	N/A	N/A
49	Paint	1y	M	Laminitis, castration surgery 7d. prior, developed diarrhea and right dorsal colitis, then laminitis	Diarrhea, Laminitis	Anesthesia / Surgery, Colic	Lung - Necropsy	B	typhimurium	N/A	N/A
50	TB	3y	M	Presented to VMTH with leukopenia & diarrhea, transfaunation x 2, started iodochlorhydroxyquin therapy, given Probios®	Diarrhea - Chronic	Antibiotic Administration	Feces	B	saint paul	N/A	N/A
51	QH	8y	M	Diarrhea	Diarrhea	None Determined	Feces	C1	mbandaka	N/A	N/A
52	TB	3m	F	Diarrhea, fever. Treated with metronidazole for diarrhea before admission	Diarrhea, Fever	Antibiotic Administration	Feces	C2	newport	D	miami
53	Paint	22y	M	Shipped from NC 2 weeks previously. Anorexia, cecal impaction.	Colic - Diarrhea	Colic, Shipping	Feces	D	miami	N/A	N/A
54	QH	2y	F	Diarrhea, fever	Diarrhea, Fever	Antibiotic Administration	Feces	B	saint paul	N/A	N/A

Table D-1. Continued

Case ID	Breed	Age	Sex	Presenting Complaint	Clinical Syndrome	Risk Factors	Specimen Origin	Salmonella Group	Serovar	2nd Salmonella Group	2nd Serovar
55	TB	1y	F	Surgery at VMTH for corneal stromal abscess, sent home on doxycycline. Diarrhea & fever 2d after discharge, treated with ceftiofur and metronidazole after fever started	Post-Op (non-colic) Diarrhea	Antibiotic Administration, Surgery/Anesthesia, Systemic Disease	SI - Necropsy	B	java	N/A	N/A
56	QH	16y	M	Chronic diarrhea, lymphocytic-plasmacytic enteritis, referred from AL for unresponsive diarrhea and hypoproteinemia, rectal biopsy negative for *Salmonella*	Diarrhea - Chronic	Colic	Feces	C2	muenchen	N/A	N/A
57	Paso Fino	1y	M	Colic, weight loss, GDUD, treated with erythromycin for suspected ileus	Colic - Diarrhea	Colic, Antibiotic Administration	Feces	B	java	N/A	N/A
58	TB	3m	F	Diarrhea, fever, treated with metronidazole for diarrhea before admission	Diarrhea, Fever	Antibiotic Administration	Feces	D	miami	C2	newport
59	Arab	3y	M	Chronic colic, current episode, SI resection 12-15ft, *Salmonella* isolated from SI at surgery. TMPS given 3m prior just before first colic episode occurred	Post-Op Colic - No Diarrhea, Chronic Colic	Colic, Anesthesia / Surgery, Antibiotic Administration	Feces / SI	B	java	N/A	N/A
60	Unknown	3y	M	Surgical patient from referral hospital		Anesthesia / Surgery	Feces	B	saint paul	N/A	N/A
61	Miniature Horse	3m	M	Congenital cataracts, cataract surgery, history of pneumonia prior to surgery	Post-Op (non-colic) Diarrhea	Anesthesia / Surgery, Systemic Disease (non-colic)	Feces	B	java	N/A	N/A

Table D-1. Continued

Case ID	Breed	Age	Sex	Presenting Complaint	Clinical Syndrome	Risk Factors	Specimen Origin	Salmonella Group	Serovar	2nd Salmonella Group	2nd Serovar
62	TB	5m	F	Diarrhea, pneumonia	Diarrhea, Pneumonia	Systemic Disease (non-colic), Antibiotic Administration	Feces	D	miami	N/A	N/A
63	TB	3m	M	Diarrhea, fever, treated with penicillin G for fever	Diarrhea, Fever	Antibiotic Administration	Feces	E	london	N/A	N/A
64	Unknown	6y	M				Biopsy - Rectal	E	meleagridis	N/A	N/A
65	QH	3y	M	Colic – right dorsal displacement	Post-Op Colic Diarrhea	Colic, Anesthesia / Surgery	Feces	D	javiana	N/A	N/A
66	TB	3m	F	Hepatic encephalopathy, endocarditis, fever, strangles recovery	Fever - HE	Systemic Disease (non-colic)	Feces	C2	newport	N/A	N/A
67	Unknown	6y	F	Fever, diarrhea	Diarrhea - Fever		Feces	D	miami	N/A	N/A
68	Pony	3m	M	Fever, diarrhea, respiratory infection	Diarrhea - Fever	Antibiotic Administration, Systemic Disease	Feces	C2	muenchen	N/A	N/A
69	TB	6y	M	Colic, large colon impaction / displacement	Post-Op Colic Diarrhea	Colic, Anesthesia / Surgery	Feces	D	javiana	B	java
70	TB	6y	M	Colic, large colon impaction / displacement	Post-Op Colic Diarrhea	Colic, Anesthesia / Surgery	Feces	B	java	D	javiana
71	Miniature Horse	3m	M	Anorexia, depression, diarrhea	Diarrhea		Feces	D	miami	B	java
72	TB	3m	M	Rotavirus +, fever, diarrhea, GDUD, colic	Colic - Diarrhea, Fever	Colic, Systemic Disease	Feces	B	saint paul	N/A	N/A
73	Miniature Horse	6y	F	Diarrhea, fever. "Every summer gets intermittent diarrhea"	Diarrhea, Fever	None Determined	Feces	C1	hartford	C2	newport
74	Paint	1.5y	M	Chronic diarrhea for 4.5m prior to presentation	Diarrhea - Chronic	None Determined	Liver - Necropsy	C2	muenchen	N/A	N/A

Table D-1. Continued

Case ID	Breed	Age	Sex	Presenting Complaint	Clinical Syndrome	Risk Factors	Specimen Origin	Salmonella Group	Serovar	2nd Salmonella Group	2nd Serovar
75	Miniature Horse	3m	M	Anorexia, depression, diarrhea	Diarrhea	None Determined	Feces	B	java	D	miami
76	Paso Fino	4m	F	Diarrhea, developed laminitis	Diarrhea, Laminitis	None Determined	Feces	C1	infantis	N/A	N/A
77	Appaloosa	2m	M	Diarrhea, fever, de-wormed the day before diarrhea started	Diarrhea - Fever	Deworming	Feces	D	miami	N/A	N/A
78	Welsh pony	18y	F	Gastric impaction (persimmon fruit), gastrotomy surgery	Post-Op Colic Diarrhea	Colic, Anesthesia / Surgery	Feces	B	java	N/A	N/A
79	Holsteiner	7-8y	F	Colic, right dorsal displacement with colonic volvulus. Isolate lost.	Post-Op Colic Diarrhea	Colic, Surgery / Anesthesia	Feces	D	javiana	N/A	N/A
80	Miniature Horse	7y	M	Fever, diarrhea, anorexia	Diarrhea, Fever	None Determined	Feces	C2	muenchen	F	rubishlaw
81	QH	3m	M	Colic (SI volvulus), chronic diarrhea 3mo.	Colic - Diarrhea, Chronic Diarrhea	Colic, Anesthesia / Surgery	Feces	B	multiple serovars (NVSL sample)	C2	muenchen
82	Paso Fino	7m	M	Colic, ileocecal intussusception - NO surgery – medically treated then euthanatized	Colic - Diarrhea	Colic	SI - Necropsy	B	saint paul	N/A	N/A
83	Unknown		M				LI - Necropsy	B	reading	N/A	N/A
84	QH	2y	F	Chronic colic, protein losing enteropathy	Chronic Colic - No Diarrhea	Colic	Feces	E	anatum	N/A	N/A
85	TB	5y	M	Epiploic foramen entrapment with jejunal resection. Leaky anastomosis and peritonitis	Post-Op Colic Diarrhea	Colic, Anesthesia / Surgery	Feces	B	java	N/A	N/A

Table D-1. Continued

Case ID	Breed	Age	Sex	Presenting Complaint	Clinical Syndrome	Risk Factors	Specimen Origin	Salmonella Group	Serovar	2nd Salmonella Group	2nd Serovar
86	TB	7m	F	Severe GDUD, 5 wks ADR, treated for prior respiratory disease	Pneumonia, Diarrhea	Antibiotic Administration, Colic, Systemic Disease	Feces	B	java	N/A	N/A
87	TB	3y	F	Chronic diarrhea, wt. loss. Treated with probiotic, gastrocote, activated charcoal, iodochlorhydroxyquin (1 dose) - no response. 4d of VSL-3 (probiotic) and treated with ICH 10g PO once daily for 14d. Neg. rectal biopsy for *Salmonella*	Diarrhea - Chronic	None Determined	Feces	C2	newport	N/A	N/A
88	TB	5y	F	Post-op gastric cannulation surgery - developed pipe-stream diarrhea, fever, depression. Treated with enrofloxacin for 24d. due to environmental risk	Post-Op (non-colic) Diarrhea, Fever	Anesthesia / Surgery	Feces	B	java	N/A	N/A
89	Unknown	6m					Feces	B	typhimurium (copenhagen)	N/A	N/A
90	Paso Fino	3y	M	Fever, diarrhea developed 5d. after starting antibiotics	Diarrhea, Fever	Antibiotic Administration	Feces	C2	tallahassee	N/A	N/A
91	Oldenburg	6y	M	Large colon impaction, shipped from NY 1 week prior	Colic - No Diarrhea	Shipping, Colic	Feces	C2	newport	N/A	N/A
92	Unknown	10y	F				Feces	B	java	N/A	N/A
93	Paso Fino	6y	F	Diarrhea, colic, pregnant (aborted fetus during hospitalization)	Colic - Diarrhea	Colic, pregnant	Feces	D	miami	N/A	N/A
94	Unknown	10y	M				Feces	E	newington	N/A	N/A

Table D-1. Continued

Case ID	Breed	Age	Sex	Presenting Complaint	Clinical Syndrome	Risk Factors	Specimen Origin	Salmonella Group	Serovar	2nd Salmonella Group	2nd Serovar
95	Miniature Horse	4y	M	2wks prior injured hind limb, given phenylbutazone and flunixin (possible overdose on phenylbutazone)	Colic - Diarrhea	Colic, Systemic Disease	Feces	C2	newport	N/A	N/A
96	Unknown	26y	M				Feces	B	java	N/A	N/A
97	QH	2y	M	Small colon impaction, rectal mucosal irritation	Post-Op Colic Diarrhea	Colic, Anesthesia / Surgery	Feces	B	java	N/A	N/A
98	Unknown	5y	M				Feces	B	java	N/A	N/A
99	Unknown		F				Feces	C2	newport	N/A	N/A
100	Unknown						Feces	E	anatum	N/A	N/A
101	Unknown	1y					SI - Necropsy	B	saint paul	N/A	N/A
102	Unknown						Feces	C2	newport	N/A	N/A
103	Unknown						Feces	E	newington	N/A	N/A
104	Unknown						Feces	C1	mbandaka	N/A	N/A
105	Unknown				Abscess		Abscess	D	unknown	N/A	N/A
106	Unknown	1y						C1	thompson	N/A	N/A
107	QH	3m	M				Feces	D	javiana	N/A	N/A
108	TB	3m	M		Diarrhea		Feces	D	unknown	N/A	N/A
109	TB	16y	F		Colic - No Diarrhea	Colic	Gastric Reflux	D	unknown	N/A	N/A
110	Unknown	6m	M				Feces	D	unknown	N/A	N/A
111	TB	8m	M				Feces	D	javiana	N/A	N/A

Table D-1. Continued

Case ID	Breed	Age	Sex	Presenting Complaint	Clinical Syndrome	Risk Factors	Specimen Origin	Salmonella Group	Serovar	2nd Salmonella Group	2nd Serovar
112	Paso Fino	1.5m	F	Chronic diarrhea. Previously admitted to VMTH 6d earlier for diarrhea (presumed sand induced). *Salmonella* recovered from physis at necropsy (isolate not saved)	Diarrhea - Chronic	Antibiotic Administration	Physis	B	typhimurium (copenhagen)	N/A	N/A
113	TB	1m	F	Shipped from IN 5d before presentation, pneumonia, polysynovitis, pericarditis, sepsis, fever	Bacteremia, Diarrhea	Shipping	Lung - Necropsy	B	typhimurium	N/A	N/A
114	TB	1m	F	Shipped from IN 5d before presentation, pneumonia, polysynovitis, pericarditis, sepsis, fever	Bacteremia, Diarrhea	Shipping	Joint Fluid	B	typhimurium	N/A	N/A
115	TB	1m	F	Shipped from IN 5d before presentation, pneumonia, polysynovitis, pericarditis, sepsis, fever	Bacteremia, Diarrhea	Shipping	Blood	B	typhimurium	N/A	N/A
116	Unknown	10y	M			Colic	Gastric Reflux	E	newington	N/A	N/A
117	Unknown	3m	F				Feces	C2	newport	N/A	N/A

Table D-2. Salmonella case descriptive information: serovar, date sample taken, presence of diarrhea, total hospitalization cost, case outcome, hospitalization days, number of positive cultures, hematologic indices at time of positive culture, and total protein changes during hospitalization. Blank cells indicate missing or unavailable records.

Case ID	Serovar	Date Sample Collected	Diarrhea?	Total Bill	Outcome	Hospital Days	# Positive Cultures Out of Total	Total WBC Count	Neutrophils	TP at Admission	TP at Death or Discharge
1	N/A	Sep-99	NO	$2,034.30	Lived	11	2/5	3590	1080	6.0	6.1
2	N/A	Dec-99	YES		Unknown						
3	typhimurium	Dec-99	YES	$3,041.50	Euthanatized	16	5/5	3600	720	6.4	5.5
4	anatum	Dec-99	YES	$4,898.15	Died	5	3/5	1800	20	7.3	6.0
5	newport	Jan-00	YES	$3,299.20	Euthanatized	5	3/5	1690	422	6.9	5.1
6	typhimurium	Feb-00	YES	$3,341.20	Lived	24	2/7	18700	13000	3.9	5.4
7	typhimurium	Mar-00	YES	$1,111.35	Euthanatized	3	1/1	14900	9830	6.5	
8	typhimurium	Apr-00	YES	$3,487.50	Lived	48	1/5	5130	3620	7.4	6.7
9	java	Apr-00	NO	$4,765.50	Euthanatized	10	1/1	13000	10730	6.4	7.8
10	4,5,12 : i-monophasic	Apr-00	YES		Died	0	2/2	7700	1900	7.1	7.1
11	4,5,12 : i-monophasic	Apr-00	NO	$1,433.10	Euthanatized	10		11300	9550	5.3	5.3
12	newport	Apr-00	YES	$547.25	Euthanatized	1		2060	0	7.4	7.2
13	java	May-00	YES		Lived	29	3/3	6190	4620	7.5	6.5
14	newport	May-00	YES		Lived	8	1/1	2700	1260	4.6	5.0
15	java	May-00	YES	$4,887.15	Lived	12	5/5	1750	720	6.2	5.2
16	java	May-00	YES	$1,869.30	Lived	9	3/4	5890	3330	5.9	6.7
17	java	May-00	YES	$2,027.20	Lived	7	5/5	1460	610	6.2	6.2
18	agona	May-00			Died	0					
19	typhimurium (copenhagen)	May-00	YES	$3,720.25	Euthanatized	13	3/4	8830	900	6.3	4.2
20	java	Jun-00	YES	$1,610.80	Lived	11	3/5	1050	60	5.8	4.7
21	4,5,12 : i-monophasic	Jun-00	YES	$1,376.15	Lived	6	1/1	3860	2100	6.7	6.2

Table D-2. Continued

Case ID	Serovar	Date Sample Collected	Diarrhea?	Total Bill	Outcome	Hospital Days	# Positive Cultures Out of Total	Total WBC Count	Neutrophils	TP at Admission	TP at Death or Discharge
22	typhimurium (copenhagen)	Jun-00	YES	$2,412.25	Euthanatized	10	4/5	5420	380	5.5	4.0
23	typhimurium	Jun-00	YES	$3,070.15	Died	8		16300	10510	5.6	3.5
24	hartford	Jun-00	YES (noted at necropsy)	$440.80	Euthanatized	3	1/1	8010	5720	7.2	
25	typhimurium	Jun-00	YES	$325.65	Euthanatized	0	1/1	12000	5400	7.7	7.7
26	typhimurium (copenhagen)	Jun-00	YES		Euthanatized	13	5/7	16300	6031	7.8	5.0
27	java	Jun-00	YES		Lived	14		5910	3690	7.0	6.2
28	java	Jun-00	YES	$1,735.70	Lived	18	4/5	9010	4770	6.3	6.3
29	java	Jun-00	YES	$8,888.60	Euthanatized	30	1/8	2100	290	7.8	3.6
30	unknown - NVSL sample contaminated	Jun-00	YES		Lived	5	1/1	4100	2110	6.2	6.5
31	javiana	Jul-00	YES	$1,000.45	Lived	5	1/5	6360	3700	7.4	6.5
32	braenderup	Jul-00	YES	$1,412.65	Lived	6	1/4	4370	310	5.5	5.0
33	java	Jul-00	NO	$4,471.80	Euthanatized	2	1/1	12400	6450	5.7	5.9
34	newport	Jul-00	YES		Lived		1/5	2060	610	5.6	4.8
35	anatum	Aug-00	YES		Lived	16	4/4	3440	2110	7.6	7.2
36	rubishlaw	Aug-00	YES		Lived	6	3/5	3070	1700	7.8	7.2
37	muenchen	Aug-00	YES		Lived		5/5	8610	4700	7.8	6.7
38	javiana	Sep-00	NO	$911.50	Lived	4	5/5	2850	1120	7.3	
39	newport	Sep-00	YES		Lived	10	2/5	2890	260	6.6	5.5
40	typhimurium (copenhagen)	Sep-00	YES		Lived	5	4/5	4260	2600	5.8	4.5
41	java	Sep-00	YES	$6,790.55	Euthanatized	9	5/5	3260	1200	6.8	5.5
42	unknown (NVSL sample contaminated)	Oct-00	YES	$1,081.70	Died	13	2/5	6330	3150	7.0	5.5

Table D-2. Continued

Case ID	Serovar	Date Sample Collected	Diarrhea?	Total Bill	Outcome	Hospital Days	# Positive Cultures Out of Total	Total WBC Count	Neutrophils	TP at Admission	TP at Death or Discharge
43	typhimurium (copenhagen)	Nov-00	YES		Lived	13	2/5	4390	640	7.2	5.8
44	typhimurium (copenhagen)	Dec-00	YES		Lived	22	1/10	3090	1100	6.0	5.8
45	london	Feb-01	YES	$834.80	Lived	5	1/1	4130	2190	6.7	6.5
46	muenchen	Feb-01			Died	0	1/1				
47	newport	Mar-01	YES	$3,278.65	Euthanatized	6	1/5	6380	3000	5.9	6.0 (w/plasma)
48	typhimurium	Apr-01	YES	$300.00	Euthanatized	1	1/1				
49	typhimurium	Apr-01	YES	$546.10	Euthanatized	1	1/1	10600	6650	5.6	5.6
50	saint paul	May-01	YES	$5,312.95	Euthanatized	8	2/10	3560	1100	6.0	6.8
51	mbandaka	May-01	YES		Lived	13	2/4	3080	230	6.2	5.6
52	newport	May-01	YES	$930.70	Lived	14	4/5	34400	28500	6.3	5.6
53	miami	May-01	YES		Lived	6	4/4	3080	990	7.7	7.3
54	saint paul	May-01	YES		Lived	4	1/5	17500	13700	6.8	6.8
55	java	Jul-01	YES	$1,128.55	Euthanatized	5	1/1	3360	100	7.1	4.7
56	muenchen	Jun-01	YES	$2,988.20	Lived	11	5/5	3490	2050	3.1	3.4/3.7
57	java	Jun-01	YES		Lived	7	1/3	5090	2400	8.0	6.7
58	miami	May-01	YES	$930.70	Lived	14	4/5	34400	28500	6.3	5.6
59	java	Jul-01	NO	$5,936.40	Lived	11	2/2	5590	3710	7.1	6.5
60	saint paul	Jul-01			Died - Unknown Cause	0					
61	java	Jul-01	YES	$7,320.40	Lived	26	5/7	4660	1490	7.0	6.0
62	miami	Jul-01	YES	$1,002.65	Lived	6	1/3	12000	6180	5.8	5.5
63	london	Jul-01	YES	$2,469.05	Lived	7	3/5	6900	700	6.3	4.7
64	meleagridis	Jul-01			Died - Unknown Cause	0					

Table D-2. Continued

Case ID	Serovar	Date Sample Collected	Diarrhea?	Total Bill	Outcome	Hospital Days	# Positive Cultures Out of Total	Total WBC Count	Neutrophils	TP at Admission	TP at Death or Discharge
65	javiana	Jul-01	YES	$2,616.70	Lived	10	1/1	4990	2850	6.1	6.0
66	newport	Aug-01	NO	$1,281.50	Euthanatized	2		15300	11020	6.9	6.7
67	miami	Aug-01	YES		Lived	15	3/5	2220	570	7.8	6.0
68	muenchen	Aug-01	YES	$1,631.10	Lived	8	2/5	2950	0	5.2	5.8
69	javiana	Aug-01	YES	$3,769.85	Lived	8	3/5	4070	2770	6.2	7.1
70	java	Aug-01	YES	$3,769.85	Lived	8	3/5	4070	2770	6.2	7.1
71	miami	Aug-01	YES	$1,453.15	Lived	5	1/5	3460	470	5.8	7.0
72	saint paul	Aug-01	YES	$3,152.35	Lived	18	1/5	13400	10180	6.2	6.0
73	hartford	Sep-00	YES		Lived	10	2/5	2890	260	6.6	5.5
74	muenchen	Sep-01	YES	$1,112.05	Euthanatized	3	0/5	3740	7400	7.0	7.4
75	java	Aug-01	YES	$1,453.15	Lived	5	1/5	3460	470	5.8	7.0
76	infantis	Sep-01	YES	$4,245.65	Lived	25	3/5	14700	12080	3.9	6.0
77	miami	Sep-01	YES	$2,143.00	Euthanatized	7	4/5	5240	50	5.6	3.5
78	java	Sep-01	YES	$9,309.10	Euthanatized	13	1/3	1730	180	6.5	5.2
79	javiana	Oct-00	YES		Lived	9	1/1	4230	2440	6.1	7.4
80	muenchen	Aug-00	YES		Lived	6	3/5	3070	1700	7.8	7.2
81	multiple serovars (NVSL sample)	Aug-00	YES		Lived		5/5	8610	4700	7.8	6.7
82	saint paul	Oct-01	YES	$1,382.90	Euthanatized	6	1/1	5360	3240	6.5	6.8
83	reading	Nov-01			Died - Unknown Cause						
84	anatum	Nov-01	NO		Lived	3	1/5	22100	14000	3.5	3.7
85	java	Nov-01	YES	$6,076.40	Euthanatized	7	1/2	2520	160	6.8	4.5
86	java	Dec-01	YES	$1,661.00	Euthanatized	7	2/2	4030	2760	6.2	6.0
87	newport	Jan-02	YES	$3,541.15	Lived	36	1/18	15200	12390	6.6	6.5
88	java		YES		Lived	14					
89	typhimurium (copenhagen)	Nov-01			Unknown						

Table D-2. Continued

Case ID	Serovar	Date Sample Collected	Diarrhea?	Total Bill	Outcome	Hospital Days	# Positive Cultures Out of Total	Total WBC Count	Neutrophils	TP at Admission	TP at Death or Discharge
90	tallahassee	Dec-01	YES	$1,470.35	Euthanatized	1	2/2	7250	260	5.3	5.6
91	newport	Dec-01	NO		Lived	4	2/2	4860	3330	6.4	8.2
92	java	Dec-01			Unknown						
93	miami	Dec-01	YES		Lived	9	1/5	1670	90	6.1	5.4
94	newington	Jan-02			Unknown						
95	newport	Jan-02	YES		Lived	13	6/8	7960	5060	5.5	5.5
96	java	Jan-02			Unknown						
97	java	Jan-02	YES		Euthanatized	7	1/5	5710	915	6.2	3.5
98	java	May-02			Unknown						
99	newport	Jun-02			Unknown						
100	anatum	Oct-02			Unknown						
101	saint paul	Oct-02			Unknown						
102	newport	Oct-02			Unknown						
103	newington	Oct-02			Unknown						
104	mbandaka	Oct-02			Unknown						
105	N/A	Mar-00	NO		Lived	0	1/1				
106	thompson	Nov-01			Unknown						
107	javiana	Jun-99			Unknown						
108	N/A	Jun-99	YES		Unknown						
109	N/A	Jun-99	NO		Unknown						
110	N/A	Oct-99			Unknown						
111	javiana	Aug-99			Unknown						
112	typhimurium (copenhagen)	Jul-00	YES		Euthanatized	13	1/1	16300	6031	7.8	5.0
113	typhimurium	Jun-00	YES	$325.65	Euthanatized	1	1/1	12000	5400	7.7	7.7
114	typhimurium	Jun-00	YES	$325.65	Euthanatized	1	1/1	12000	5400	7.7	7.7

Table D-2. Continued

Case ID	Serovar	Date Sample Collected	Diarrhea?	Total Bill	Outcome	Hospital Days	# Positive Cultures Out of Total	Total WBC Count	Neutrophils	TP at Admission	TP at Death or Discharge
115	typhimurium	Jun-00	YES	$325.65	Euthanatized	1	1/1	12000	5400	7.7	7.7
116	newington	Jan-02			Unknown						
117	newport	May-02			Unknown						
MEANS		**Jan-Mar = 13 Apr-Jun = 42 Jul-Sep = 37 Oct-Dec = 24**	**22 Unkn 11 No 84 Yes**	**$2623.23**		**9.25**	**67.93%**	**7270.11**	**4032.12**	**6.45**	**5.93**

Table D-3. Salmonella case descriptive information: serovar, antibiotic therapy prior to admission and types, antibiotic therapy during hospitalization and types. Drugs in boldface type were used specifically to treat the salmonella infection. Blank cells indicate missing or unavailable records, and three dashes indicates that nothing could be determined from the record.

Case ID	Serovar	Antibiotic Therapy Prior to Admission?	Pre-Hospitalization Antibiotic(s)	Antibiotic Therapy During Hospitalization?	Hospitalization Antibiotics
1	N/A	No	---	Yes	**Enrofloxacin** Metronidazole
2	N/A				
3	typhimurium	No	---	Yes	Metronidazole Cefazolin Gentamicin **Enrofloxacin**
4	anatum	No	---	Yes	Gentamicin Cefazolin Metronidazole
5	newport	Undetermined		Yes	Cefazolin Gentamicin Metronidazole
6	typhimurium	No	---	Yes	Ceftiofur **Enrofloxacin**
7	typhimurium	Undetermined		Yes	Amikacin Cefazolin
8	typhimurium	No	---	Yes	Metronidazole **Enrofloxacin**
9	java	Undetermined		Yes	Cefazolin Gentamicin
10	4,5,12 : i-monophasic	No	---	No	---
11	4,5,12 : i-monophasic	Yes	Trimethoprim Sulfamethoxazole Rifampin Cefazolin Azithromycin	Yes	Cefazolin Gentamicin
12	newport	Undetermined		Yes	Cefazolin Metronidazole

Table D-3. Continued

Case ID	Serovar	Antibiotic Therapy Prior to Admission?	Pre-Hospitalization Antibiotic(s)	Antibiotic Therapy During Hospitalization?	Hospitalization Antibiotics
13	java	Undetermined		Yes	Cefazolin Gentamicin Metronidazole **Enrofloxacin** Trimethoprim Sulfamethoxazole
14	newport	Yes	Penicillin Trimethoprim Sulfamethoxazole	Yes	Gentamicin Metronidazole Ampicillin **Enrofloxacin**
15	java	Yes	Penicillin	Yes	Metronidazole Cefazolin Gentamicin **Enrofloxacin**
16	java	Yes		Yes	Cefazolin Gentamicin Metronidazole Ceftiofur
17	java	Yes	Trimethoprim Sulfamethoxazole Metronidazole	Yes	Metronidazole
18	agona				
19	typhimurium (copenhagen)	Yes	Penicillin	Yes	Metronidazole Cefazolin Gentamicin **Enrofloxacin**
20	java	Yes	Gentamicin Cefazolin Metronidazole	Yes	Gentamicin Cefazolin Metronidazole
21	4,5,12 : i-monophasic	Undetermined		Yes	Ampicillin Metronidazole Amikacin

Table D-3. Continued

Case ID	Serovar	Antibiotic Therapy Prior to Admission?	Pre-Hospitalization Antibiotic(s)	Antibiotic Therapy During Hospitalization?	Hospitalization Antibiotics
22	typhimurium (copenhagen)	No	---	Yes	Cefazolin Gentamicin Metronidazole **Enrofloxacin**
23	typhimurium	Undetermined		Yes	Gentamicin Penicillin Metronidazole **Enrofloxacin**
24	hartford	Yes	Amikacin Penicillin	No	---
25	typhimurium	No	---	No	---
26	typhimurium (copenhagen)	Yes	Metronidazole	Yes	Cefazolin Metronidazole Gentamicin **Enrofloxacin**
27	java	Yes	Trimethoprim Sulfamethoxazole Cefazolin Metronidazole Gentamicin	Yes	Cefazolin Metronidazole Gentamicin **Enrofloxacin**
28	java	Yes	Trimethoprim Sulfamethoxazole	Yes	Cefazolin Gentamicin
29	java	Yes	Gentamicin	Yes	Cefazolin Gentamicin **Enrofloxacin** Metronidazole
30	unknown - NVSL sample contaminated	Undetermined		Yes	Cefazolin Gentamicin Metronidazole
31	javiana	Undetermined		No	---
32	braenderup	Yes	Gentamicin Amikacin	Yes	Metronidazole
33	java	No	---	Undetermined	Cefazolin Gentamicin

Table D-3. Continued

Case ID	Serovar	Antibiotic Therapy Prior to Admission?	Pre-Hospitalization Antibiotic(s)	Antibiotic Therapy During Hospitalization?	Hospitalization Antibiotics
34	newport	Undetermined		No	---
35	anatum	Undetermined		Yes	Metronidazole Ceftiofur
36	rubishlaw	No	---	Yes	Cefazolin Gentamicin
37	muenchen	Undetermined		Yes	Penicillin Gentamicin Metronidazole
38	javiana	Undetermined		No	---
39	newport	Undetermined		Yes	Metronidazole
40	typhimurium (copenhagen)	Yes	Trimethoprim Sulfamethoxazole Metronidazole Penicillin Gentamicin	Yes	Metronidazole
41	java	Undetermined		Yes	Penicillin Gentamicin Metronidazole
42	unknown (NVSL sample contaminated)	Yes	Trimethoprim Sulfamethoxazole Erythromycin Rifampin Metronidazole Gentamicin	Yes	**Enrofloxacin**
43	typhimurium (copenhagen)	Yes	Trimethoprim Sulfamethoxazole	Yes	Metronidazole Penicillin Amikacin
44	typhimurium (copenhagen)	Yes	Gentamicin Penicillin	Yes	Metronidazole Penicillin Amikacin
45	london	Undetermined		No	---
46	muenchen	Undetermined			
47	newport	Undetermined		Yes	Penicillin Gentamicin Metronidazole

Table D-3. Continued

Case ID	Serovar	Antibiotic Therapy Prior to Admission?	Pre-Hospitalization Antibiotic(s)	Antibiotic Therapy During Hospitalization?	Hospitalization Antibiotics
48	typhimurium	Undetermined		No	---
49	typhimurium	Yes	Tetracycline	No	---
50	saint paul	Yes	Enrofloxacin Metronidazole Penicillin	Yes	**Enrofloxacin** Metronidazole
51	mbandaka	Undetermined		Yes	**Enrofloxacin** Metronidazole
52	newport	Yes	Metronidazole	Yes	Penicillin Gentamicin Metronidazole Rifampin
53	miami	Undetermined		Yes	Gentamicin Penicillin Metronidazole
54	saint paul	Yes	Metronidazole	Yes	Metronidazole
55	java	Yes	Doxycycline	Yes	Gentamicin Metronidazole Penicillin
56	muenchen	Yes	Metronidazole	Yes	**Enrofloxacin**
57	java	No	---	No	---
58	miami	Yes	Metronidazole	Yes	Penicillin Gentamicin Metronidazole Rifampin
59	java	Yes	Trimethoprim Sulfamethoxazole	Yes	Penicillin Gentamicin **Enrofloxacin**
60	saint paul				
61	java	Yes	Ceftiofur	Yes	Trimethoprim Sulfamethoxazole Penicillin Ceftiofur Amikacin

Table D-3. Continued

Case ID	Serovar	Antibiotic Therapy Prior to Admission?	Pre-Hospitalization Antibiotic(s)	Antibiotic Therapy During Hospitalization?	Hospitalization Antibiotics
62	miami	Yes	Penicillin Gentamicin Rifampin	Yes	Penicillin Gentamicin Metronidazole **Enrofloxacin**
63	london	Yes	Penicillin	Yes	Metronidazole Gentamicin Penicillin
64	meleagridis				
65	javiana	No	---	Yes	Gentamicin Penicillin
66	newport	Yes	Penicillin Gentamicin	Yes	Penicillin Metronidazole Ceftiofur
67	miami			Yes	Penicillin Gentamicin Metronidazole **Enrofloxacin**
68	muenchen	Yes		Yes	Metronidazole Gentamicin Penicillin
69	javiana	No	---	Yes	Penicillin Gentamicin
70	java	No	---	Yes	Penicillin Gentamicin
71	miami	Undetermined		Yes	Penicillin Metronidazole Amikacin
72	saint paul	Undetermined		Yes	Metronidazole Trimethoprim Sulfamethoxazole **Enrofloxacin**
73	hartford	Undetermined		Yes	Metronidazole
74	muenchen	Undetermined		Yes	Metronidazole

Table D-3. Continued

Case ID	Serovar	Antibiotic Therapy Prior to Admission?	Pre-Hospitalization Antibiotic(s)	Antibiotic Therapy During Hospitalization?	Hospitalization Antibiotics
75	java	Undetermined		Yes	Penicillin Metronidazole Amikacin
76	infantis	Undetermined		Yes	**Enrofloxacin** Metronidazole Penicillin Gentamicin
77	miami	No	---	Yes	Penicillin Gentamicin Metronidazole
78	java	No	---	Yes	Penicillin Gentamicin **Enrofloxacin** Metronidazole
79	javiana	Undetermined		Yes	Penicillin Gentamicin
80	muenchen	No	---	Yes	Cefazolin Gentamicin
81	multiple serovars (NVSL sample)	Undetermined		Yes	Penicillin Gentamicin Metronidazole
82	saint paul	Undetermined		Yes	Penicillin Gentamicin
83	reading				
84	anatum	No	---	No	---
85	java	Undetermined		Yes	
86	java	Yes	Gentamicin Penicillin Ceftiofur	No	
87	newport	No	---	Yes	Metronidazole **Enrofloxacin**
88	java	No	---	Yes	**Enrofloxacin**

Table D-3. Continued

Case ID	Serovar	Antibiotic Therapy Prior to Admission?	Pre-Hospitalization Antibiotic(s)	Antibiotic Therapy During Hospitalization?	Hospitalization Antibiotics
89	typhimurium (copenhagen)				
90	tallahassee	Yes	Penicillin G Gentamicin	Yes	Metronidazole
91	newport	No	---	No	---
92	java				
93	miami			Yes	Gentamicin Penicillin Metronidazole
94	newington				
95	newport	No	---	Yes	Metronidazole **Enrofloxacin**
96	java				
97	java	Undetermined		Yes	Gentamicin Penicillin Metronidazole
98	java				
99	newport				
100	anatum				
101	saint paul				
102	newport				
103	newington				
104	mbandaka				
105	N/A				
106	thompson				
107	javiana				
108	N/A				
109	N/A				
110	N/A				

Table D-3. Continued

Case ID	Serovar	Antibiotic Therapy Prior to Admission?	Pre-Hospitalization Antibiotic(s)	Antibiotic Therapy During Hospitalization?	Hospitalization Antibiotics
111	javiana				
112	typhimurium (copenhagen)	Yes	Metronidazole	Yes	Cefazolin Metronidazole Gentamicin **Enrofloxacin**
113	typhimurium	No	---	No	---
114	typhimurium	No	---	No	---
115	typhimurium	No	---	No	---
116	newington				
117	newport				

APPENDIX E
SALMONELLA ISOLATE ANTIMICROBIAL SUSCEPTIBILITY DATA

Tables E1 through E3 detail all of the *in vitro* susceptibility data for 108 salmonella isolates obtained from horses.

Table E-1. Salmonella isolate MIC antibiotic sensitivity profiles. Blank cells indicate missing data. Legend: AMI = amikacin, AMOX = amoxicillin-clavulanic acid, AMP = ampicillin, CEFA = cefazolin, CEFZ = ceftazidime, NAX = ceftiofur, CHLP = chloramphenicol.

Case ID	MIC AMI	AMI	MIC AMOX	AMOX	MIC AMP	AMP	MIC CEFA	CEFA	MIC CEFZ	CEFZ	MIC NAX	NAX	MIC CHLP	CHLP
1	<=2	S	8	S	2	S	4	S	0.5000	S	<=0.5000	S	8	S
2	<=2	S	<=2	S	2	S	<=2	S	0.5000	S	<=0.5000	S	8	S
3	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	1	S	8	S
4	<=2	S	<=2	S	2	S	<=2	S	0.5000	S	1	S	8	S
5	<=2	S	<=2	S	2	S	<=2	S	0.5000	S	1	S	8	S
6	<=2	S	<=2	S	4	S	<=2	S	0.5000	S	1	S	16	I
7	<=2	S	<=2	S	2	S	<=2	S	0.5000	S	1	S	8	S
8	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.2500	S	<=4	S
13	<=2	S	>16	R	>16	R	>16	R	32	R	>4	R	<=4	S
14	<=2	S	>16	R	>16	R	>16	R	32	R	>4	R	>32	R
15	<=2	S	>16	R	>16	R	>16	R	16	I	>4	R	<=4	S
16	<=2	S	>16	R	>16	R	>16	R	32	R	>4	R	<=4	S
17	<=2	S	>16	R	>16	R	>16	R	32	R	>4	R	<=4	S
19	4	S	>16	R	>16	R	>16	R	32	R	>4	R	>32	R
20	<=2	S	>16	R	>16	R	>16	R	32	R	>4	R	<=4	S
21	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	8	S
22	<=2	S	>16	R	>16	R	>16	R	32	R	>4	R	>32	R
23	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	8	S
24	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	<=0.5000	S	8	S
25	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
26	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
27	4	S	>16	R	>16	R	>16	R	32	R	>4	R	<=4	S
28	<=2	S			>16	R					>4	R		
29	<=2	S	>16	R	>16	R	>16	R	16	I	>4	R	>32	R
30	<=2	S	>16	R	>16	R	>16	R	16	I	>4	R	<=4	S
31	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	<=0.5000	S	8	S
32	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	8	S
33	<=2	S	>16	R	>16	R	>16	R	32	R	>4	R	<=4	S
34	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
35	<=2	S	<=2	S	1	S	<=2	S	0.5000	S	<=0.5000	S	8	S

Table E-1. Continued

Case ID	MIC AMI	AMI	MIC AMOX	AMOX	MIC AMP	AMP	MIC CEFA	CEFA	MIC CEFZ	CEFZ	MIC NAX	NAX	MIC CHLP	CHLP
36	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	8	S
37	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
38	<=2	S	<=2	S	1	S	<=2	S	4	S	<=0.5000	S	<=4	S
39	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
40	<=2	S	>16	R	>16	R	>16	R	32	R	>4	R	>32	R
41	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
42	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
43	<=2	S	>16	R	>16	R	>16	R	32	R	>4	R	>32	R
44	<=2	S	>16	R	>16	R	>16	R	32	R	>4	R	>32	R
45	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
46	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
47	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	1	S	8	S
48	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
49	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
50	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
51	<=2	S	<=2	S	1	S	<=2	S	0.5000	S	<=0.5000	S	8	S
52	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
53	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
54	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
55	4	S	>16	R	>16	R	>16	R	32	R	>4	R	8	S
56	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
57	>16	R	>16	R	>16	R	>16	R	32	R	>4	R	<=4	S
58	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
59	<=2	S	>16	R	>16	R	>16	R	16	I	>4	R	<=4	S
60	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	<=0.5000	S	8	S
61	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
62	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	<=0.5000	S	8	S
63	4	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
64	<=2	S	<=2	S	2	S	<=2	S	0.5000	S	<=0.5000	S	<=4	S
65	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
66	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
67	<=2	S	<=2	S	2	S	<=2	S	0.5000	S	<=0.5000	S	<=4	S
68	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
69	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S

Table E-1. Continued

Case ID	MIC AMI	AMI	MIC AMOX	AMOX	MIC AMP	AMP	MIC CEFA	CEFA	MIC CEFZ	CEFZ	MIC NAX	NAX	MIC CHLP	CHLP
70	<=2	S	>16	R	>16	R	>16	R	16	I	>4	R	<=4	S
71	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	<=0.5000	S	8	S
72	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	<=0.5000	S	8	S
73	<=2	S	<=2	S	0.5000	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
74	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
75	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
76	<=2	S	<=2	S	2	S	<=2	S	0.5000	S	<=0.5000	S	8	S
77	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
78	<=2	S	>16	R	>16	R	>16	R	32	R	>4	R	32	R
79	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
81	<=2	S	<=2	S	1	S	<=2	S	0.5000	S	<=0.5000	S	8	S
83	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	8	S
84	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
84	<=2	S	<=2	S	1	S	<=2	S	0.5000	S	<=0.5000	S	8	S
85	<=2	S	>16	R	>16	R	>16	R	16	I	>4	R	<=4	S
86	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5	S	8	S
87	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5	S	<=4	S
89	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	1	S	<=4	S
90	<=2	S	<=2	S	2	S	<=2	S	<=0.2500	S	1	S	8	S
91	4	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.500	S	<=4	S
92	<=2	S	>16	R	>16	R	>16	R	16	I	>4	R	<=4	S
93	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.500	S	<=4	S
94	<=2	S	<=2	S	1	S	<=2	S	0.5000	S	1	S	<=4	S
95	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5	S	<=4	S
96	<=2	S	>16	R	>16	R	>16	R	16	I	>4	R	<=4	S
97	<=2	S	>16	R	>16	R	>16	R	16	I	>4	R	<=4	S
98	<=2	S	>16	R	>16	R	>16	R	16	I	>4	R	<=4	S
99	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5	S	<=4	S
100	<=2	S			<=1	S	<=2	S	<=2	S		S	<=8	S
101	<=2	S			2	S	<=2	S	<=2	S			<=8	S
102	8				<=1	S	<=2	S	<=2	S		S	<=8	S
103	8				2	S	<=2	S	<=2	S		S	<=8	S
104	8				<=1	S	<=2	S	<=1	S		S	<=8	S
105	>16	R	>16	R	>16	R	8	S	8	S	1	S	>32	R

Table E-1. Continued

Case ID	MIC AMI	AMI	MIC AMOX	AMOX	MIC AMP	AMP	MIC CEFA	CEFA	MIC CEFZ	CEFZ	MIC NAX	NAX	MIC CHLP	CHLP
106	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
107	>16	R	>16	R	>16	R	16	I	32	R	>4	R	>32	R
108	>16	R	>16	R	>16	R	>16	R	>32	R	>4	R	>32	R
109	>16	R	>16	R	>16	R	16	I	32	R	>4	R	>32	R
110	>16	R	>16	R	>16	R	>16	R	32	R	>4	R	>32	R
111	>16	R	>16	R	>16	R	>16	R	>32	R	>4	R	>32	R
115	<=2	S	<=2	S	1	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
117	<=2	S	<=2	S	1	S	<=2	S	<=0.25	S	<=0.5	S	<=4	S
118	<=2	S	<=2	S	0.5000	S	<=2	S	<=0.2500	S	<=0.5000	S	<=4	S
119	<=2	S	>16	R	>16	R	>16	R	16	I	>4	R	<=4	S

Table E-2. Salmonella isolate MIC antibiotic sensitivity profiles. Blank cells indicate missing data. Legend: CLIN = clindamycin, DOX = doxycycline, ENRO = enrofloxacin, ERYT = erythromycin, GENT = gentamicin, IMIP = imipenem.

Case ID	MIC CLIN	CLIN	MIC DOX	DOX	MIC ENRO	ENRO	MIC ERYT	ERYT	MIC GENT	GENT	MIC IMIP	IMIP
1	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
2	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
3	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
4	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
5	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
6	>2	R	>4	R	<=0.2500	S	>4	R	<=1	S	<=1	S
7	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
8	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
13	>2	R	>4	R	<=0.2500	S	>4	R	4	S	<=1	S
14	>2	R	>4	R	<=0.2500	S	>4	R	<=1	S	<=1	S
15	>2	R	>4	R	<=0.2500	S	>4	R	8	I	<=1	S
16	>2	R	>4	R	<=0.2500	S	>4	R	8	I	<=1	S
17	>2	R	>4	R	<=0.2500	S	>4	R	4	S	<=1	S
19	>2	R	>4	R	<=0.2500	S	>4	R	>8	R	<=1	S
20	>2	R	>4	R	<=0.2500	S	>4	R	8	I	<=1	S
21	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
22	>2	R	>4	R	<=0.2500	S	>4	R	>8	R	<=1	S
23	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
24	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
25	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
26	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
27	>2	R	>4	R	<=0.2500	S	>4	R	>8	R	<=1	S
28					<=0.2500	S			4	S		
29	>2	R	>4	R	<=0.2500	S	>4	R	>8	R	<=1	S
30	>2	R	>4	R	<=0.2500	S	>4	R	4	S	<=1	S
31	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
32	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
33	>2	R	>4	R	<=0.2500	S	>4	R	8	I	<=1	S
34	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
35	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
36	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S

Table E-2. Continued

Case ID	MIC CLIN	CLIN	MIC DOX	DOX	MIC ENRO	ENRO	MIC ERYT	ERYT	MIC GENT	GENT	MIC IMIP	IMIP
37	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
38	<=0.2500	S	>4	R	<=0.2500	S	<=0.2500	S	<=1	S	<=1	S
39	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
40	>2	R	>4	R	<=0.2500	S	>4	R	>8	R	<=1	S
41	>2	R	>4	R	<=0.2500	S	>4	R	8	I	<=1	S
42	>2	R	4	S	<=0.2500	S	>4	R	4	S	<=1	S
43	>2	R	>4	R	<=0.2500	S	>4	R	>8	R	<=1	S
44	>2	R	>4	R	<=0.2500	S	>4	R	>8	R	<=1	S
45	>2	R	>4	R	<=0.2500	S	>4	R	<=1	S	<=1	S
46	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
47	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
48	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
49	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
50	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
51	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
52	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
53	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
54	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
55	>4	R	>4	R	<=0.2500	S	>4	R	8	I	<=1	S
56	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
57	>2	R	>4	R	0.5000	S	>4	R	>8	R	2	S
58	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
59	>2	R	>4	R	<=0.2500	S	>4	R	4	S	<=1	S
60	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
61	>2	R	>4	R	<=0.2500	S	>4	R	8	I	<=1	S
62	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
63	>2	R	>4	R	<=0.2500	S	>4	R	<=1	S	<=1	S
64	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
65	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
66	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
67	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
68	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
69	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
70	>2	R	>4	R	<=0.2500	S	>4	R	8	I	<=1	S

Table E-2. Continued

Case ID	MIC CLIN	CLIN	MIC DOX	DOX	MIC ENRO	ENRO	MIC ERYT	ERYT	MIC GENT	GENT	MIC IMIP	IMIP
71	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
72	>2	R	>4	R	<=0.2500	S	>4	R	4	S	<=1	S
73	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
74	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
75	>2	R	>4	R	<=0.2500	S	>4	R	2	S	<=1	S
76	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
77	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
78	>2	R	>4	R	1	I	>4	R	>8	R	<=1	S
79	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
81	>2	R	>4	R	<=0.2500	S	>4	R	<=1	S	<=1	S
83	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
84	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
84	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
85	>2	R	>4	S	<=0.2500	S	>4	R	8	I	<=1	S
86	>2	R	>4	R	<=0.2500	S	>4	R	4	S	<=1	S
87	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
89	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
90	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
91	>2	R	>4	R	<=0.2500	S	>4	R	<=1	S	<=1	S
92	>2	R	>4	R	<=0.2500	S	>4	R	4	S	<=1	S
93	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
94	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
95	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
96	>2	R	>4	R	<=0.2500	S	>4	R	8	I	<=1	S
97	>2	R	>4	R	<=0.2500	S	>4	R	4	S	<=1	S
98	>2	R	>4	R	<=0.2500	S	>4	R	8	I	<=1	S
99	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
100						S			<=1	S	<=1	S
101						S			<=1	S	<=1	S
102						S			<=1	S	<=1	S
103						S			<=1	S	<=1	S
104						S			<=1	S	<=1	S
105	>2	R	>4	R	<=0.2500	S	>4	R	>8	R	<=1	S
106	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S

Table E-2. Continued

Case ID	MIC CLIN	CLIN	MIC DOX	DOX	MIC ENRO	ENRO	MIC ERYT	ERYT	MIC GENT	GENT	MIC IMIP	IMIP
107	>2	R	>4	R	<=0.2500	S	>4	R	>8	R	<=1	S
108	>2	R	>4	R	<=0.2500	S	>4	R	>8	R	<=1	S
109	>2	R	>4	R	<=0.2500	S	>4	R	>8	R	<=1	S
110	>2	R	>4	R	<=0.2500	S	>4	R	>8	R	<=1	S
111	>2	R	>4	R	<=0.2500	S	>4	R	>8	R	<=1	S
115	>2	R	4	S	<=0.2500	S	>4	R	<=1	S	<=1	S
117	>2	R	2	S	<=0.2500	S	>4	R	<=1	S	<=1	S
118	>2	R	1	S	<=0.2500	S	>4	R	<=1	S	<=1	S
119	>2	R	>4	R	<=0.2500	S	>4	R	4	S	<=1	S

Table E-3. Salmonella isolate MIC antibiotic sensitivity profiles. Blank cells indicate missing data. Legend: NITR = nitrofurantoin, OX = oxacillin, PEN = penicillin, RIF = rifampin, TET = tetracycline, TMP = trimethoprim-sulfamethoxazole.

Case ID	MIC NITR	NITR	MIC OX	OX	MIC PEN	PEN	MIC RIF	RIF	MIC TET	TET	MIC TMP	TMP
1	<=32	S	>4	R	>16	R	>4	R	<=2	S	<=0.2500	S
2	<=32	S	>4	R	8	R	4	R	<=2	S	<=0.2500	S
3	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
4	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
5	<=32	S	>4	R	16	R	>4	R	<=2	S	<=0.2500	S
6	<=32	S	>4	R	16	R	>4	R	4	S	<=0.2500	S
7	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
8	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
13	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
14	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
15	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
16	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
17	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
19	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
20	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
21	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
22	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
23	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
24	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
25	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.25	S
26	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
27	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
28											>4	R
29	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
30	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
31	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
32	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
33	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
34	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
35	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
36	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
37	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S

Table E-3. Continued

Case ID	MIC NITR	NITR	MIC OX	OX	MIC PEN	PEN	MIC RIF	RIF	MIC TET	TET	MIC TMP	TMP
38	<=32	S	<=2	S	8	R	<=0.5000	S	>16	R	>4	R
39	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.25	S
40	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
41	<=32	S	>4	R	4	R	>4	R	>16	R	>4	R
42	<=32	S	>4	R	8	R	>4	R	<=2	S	>4	R
43	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
44	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
45	<=32	S	>4	R	8	R	>4	R	>16	R	<=0.2500	S
46	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
47	<=32	S	>4	R	16	R	>4	R	<=2	S	<=0.2500	S
48	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
49	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
50	<=32	S	>4	R	8	R	4	R	<=2	S	<=0.2500	S
51	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
52	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
53	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
54	<=32	S	>4	R	16	R	>4	R	<=2	S	<=0.2500	S
55	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
56	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
57	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
58	<=32	S	>4	R	8	R	4	R	<=2	S	<=0.2500	S
59	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
60	<=32	S	>4	R	16	R	>4	R	<=2	S	<=0.25	S
61	<=32	S	>4	R	16	R	>4	R	>16	R	>4	R
62	<=32	S	>4	R	16	R	>4	R	<=2	S	<=0.2500	S
63	<=32	S	>4	R	8	R	>4	R	>16	R	<=0.2500	S
64	<=32	S	>4	R	16	R	>4	R	<=2	S	<=0.25	S
65	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
66	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
67	<=32	S	>4	R	16	R	>4	R	<=2	S	<=0.2500	S
68	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
69	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
70	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
71	<=32	S	>4	R	16	R	>4	R	<=2	S	<=0.2500	S

Table E-3. Continued

Case ID	MIC NITR	NITR	MIC OX	OX	MIC PEN	PEN	MIC RIF	RIF	MIC TET	TET	MIC TMP	TMP
72	<=32	S	>4	R	16	R	>4	R	>16	R	>4	R
73	<=32	S	>4	R	4	R	>4	R	<=2	S	<=0.2500	S
74	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
75	<=32	S	>4	R	8	R	>4	R	>16	R	>4	R
76	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
77	<=32	S	>4	R	16	R	>4	R	<=2	S	<=0.2500	S
78	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
79	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
81	<=32	S	>4	R	8	R	>4	R	>16	R	<=0.25	S
83	<=32	S	>4	R	8	R	>4	R	<=2	S	0.5	S
84	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
84	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
85	<=32	S	>4	R	>16	R	4	R	>16	R	>4	R
86	<=32	S	>4	R	16	R	>4	R	>16	R	>4	R
87	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
89	<=32	S	>4	R	16	R	>4	R	<=2	S	<=0.2500	S
90	<=32	S	>4	R	>16	R	>4	R	<=2	S	<=0.2500	S
91	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
92	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
93	<=32	S	>4	R	16	R	>4	R	<=2	S	<=0.2500	S
94	<=32	S	>4	R	16	R	>4	R	<=2	S	<=0.2500	S
95	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
96	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
97	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
98	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
99	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
100	<=32	S							<=4	S	<=0.0530	S
101	<=32	S							>8	R	>0.0530	R
102	<=32	S							<=4	S	<=0.0625	S
103	<=32	S							<=4	S	<=0.0530	S
104	<=32	S							<=4	S	<=0.0530	S
105	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
106	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
107	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R

Table E-3. Continued

Case ID	MIC NITR	NITR	MIC OX	OX	MIC PEN	PEN	MIC RIF	RIF	MIC TET	TET	MIC TMP	TMP
108	<=32	S	>4	R	>16		>4	R	>16	R	>4	R
109	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
110	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
111	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R
115	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
117	<=32	S	>4	R	8	R	>4	R	<=2	S	<=0.2500	S
118	<=32	S	>4	R	2	R	>4	R	<=2	S	<=0.2500	S
119	<=32	S	>4	R	>16	R	>4	R	>16	R	>4	R

APPENDIX F
DESCRIPTIVE STATISTICS

Table F-1. Descriptive statistics for individual cecal SCFA measurements before and after four days of control (no) antibiotic treatment in 5 horses

	N	Mean	SD	Minimum	Maximum
PRE Acetate	5	4.0320	.77380	3.20	5.04
PRE Propionate	5	3.6280	2.64455	1.35	6.67
PRE Isobutyrate	5	5.8700	2.40677	4.18	9.94
PRE Butyrate	5	7.0700	5.23751	3.41	15.86
PRE Isovalerate	5	2.8380	.29406	2.53	3.22
PRE Valerate	5	5.4020	4.04363	2.27	11.27
PRE Ethyl butyrate	5	8.5560	2.20525	5.96	11.64
POST Acetate	5	4.9700	1.00045	4.04	6.30
POST Propionate	5	4.2840	1.60690	2.70	5.97
POST Isobutyrate	5	6.9080	1.36280	5.57	9.03
POST Butyrate	5	8.3640	3.25675	5.46	13.72
POST Isovalerate	5	3.5980	.50549	3.24	4.46
POST Valerate	5	6.3180	2.93807	3.86	10.15
POST Ethyl butyrate	5	9.6140	.82494	8.61	10.78

Table F-2. Descriptive statistics for individual cecal SCFA measurements in 5 horses treated with intramuscular ceftiofur sodium at 2 mg/kg twice daily, before and after 4 days of treatment

	N	Mean	SD	Minimum	Maximum
PRE Acetate	5	5.0220	.93977	4.38	6.67
PRE Propionate	5	5.2960	1.63476	3.01	7.52
PRE Isobutyrate	5	7.2480	.74530	6.40	8.39
PRE Butyrate	5	10.8360	3.37304	6.04	15.07
PRE Isovalerate	5	3.9200	.71186	2.99	4.96
PRE Valerate	5	7.5140	3.04736	4.00	11.41
PRE Ethyl butyrate	5	10.3000	3.92519	7.02	17.09
POST Acetate	5	4.9300	.69487	4.34	6.11
POST Propionate	5	5.3920	1.14018	4.39	7.15
POST Isobutyrate	5	7.1960	.99405	5.94	8.31
POST Butyrate	5	10.9320	2.56456	6.91	13.88
POST Isovalerate	5	3.9920	.76405	3.14	5.04
POST Valerate	5	6.9860	1.79723	4.08	8.35
POST Ethyl butyrate	5	8.3860	.89985	7.12	9.47

Table F-3. Descriptive statistics for individual cecal SCFA measurements in 5 horses treated with intravenous oxytetracycline at 10 mg/kg once daily, before and after 4 days of treatment

	N	Mean	SD	Minimum	Maximum
PRE Acetate	5	4.6000	.74310	4.09	5.86
PRE Propionate	5	4.5640	2.43428	1.86	8.40
PRE Isobutyrate	5	6.7180	1.06528	5.38	8.10
PRE Butyrate	5	9.4980	4.82499	3.51	16.41
PRE Isovalerate	5	3.4680	.27216	3.14	3.78
PRE Valerate	5	6.4320	3.00215	3.10	10.38
PRE Ethyl butyrate	5	8.1620	.68460	7.71	9.27
POST Acetate	5	4.6080	.36128	4.16	5.12
POST Propionate	5	5.3760	1.90697	3.68	8.65
POST Isobutyrate	5	6.9340	1.21829	5.09	8.14
POST Butyrate	5	10.9120	3.55481	8.40	17.02
POST Isovalerate	5	3.9820	.99117	2.70	5.32
POST Valerate	5	7.2620	1.89179	5.73	10.26
POST Ethyl butyrate	5	7.2400	.83979	6.56	8.67

Table F-4. Descriptive statistics for individual cecal SCFA measurements in 5 horses treated with oral trimethoprim-sulfamethoxazole at 30 mg/kg twice daily, before and after 4 days of treatment

	N	Mean	SD	Minimum	Maximum
PRE Acetate	5	4.3060	.81488	3.41	5.49
PRE Propionate	5	4.4360	.77957	3.64	5.50
PRE Isobutyrate	5	6.6060	1.64246	4.74	9.00
PRE Butyrate	5	9.4860	1.97744	6.90	12.40
PRE Isovalerate	5	4.0620	1.51098	2.55	6.50
PRE Valerate	5	6.2380	1.86963	5.04	9.56
PRE Ethyl butyrate	5	7.6380	1.62737	5.28	9.85
POST Acetate	5	4.4400	.21966	4.18	4.76
POST Propionate	5	3.7860	1.48702	2.46	6.09
POST Isobutyrate	5	6.3920	1.24638	4.83	7.86
POST Butyrate	5	7.4420	2.93952	4.93	12.26
POST Isovalerate	5	3.8760	.68937	2.84	4.58
POST Valerate	5	5.6360	2.86201	3.80	10.67
POST Ethyl butyrate	5	9.2860	1.93660	6.99	11.00

Table F-5. Descriptive statistics for cecal protozoal counts in 5 horses treated with control (no treatment), ceftiofur sodium, oxytetracycline, or trimethoprim-sulfamethoxazole, before and after 4 days of treatment

Time and Treatment	N	Mean	SD	Minimum	Maximum
PRE Control	5	1533.2000	410.20385	966.00	2066.00
PRE Ceftiofur	5	6050.0000	4463.93392	1534.00	11950.00
PRE Oxytetracycline	5	4626.8000	4525.97715	716.00	10150.00
PRE TMPS	5	4320.8000	2842.31195	616.00	8566.00
POST Control	5	2946.8000	3049.84905	516.00	8100.00
POST Ceftiofur	5	5736.4000	2110.61005	4534.00	9500.00
POST Oxytetracycline	5	5960.0000	3484.33006	1966.00	10934.00
POST TMPS	5	5343.6000	4860.91317	1900.00	13650.00

Table F-6. Descriptive statistics for cecal pH in 5 horses treated with control (no treatment), ceftiofur sodium, oxytetracycline, or trimethoprim-sulfamethoxazole, before and after 4 days of treatment

	N	Range	Minimum	Maximum	Mean	SE	SD	Var
PRE Control	5	.837	6.103	6.940	6.33780	.15278	.341637	.117
POST Control	5	.747	5.743	6.490	6.10200	.11833	.264592	.070
PRE Ceftiofur	5	.573	6.560	7.133	6.74240	.10717	.239628	.057
POST Ceftiofur	5	.545	6.605	7.150	6.83500	.10305	.230434	.053
PRE TET	5	.530	6.327	6.857	6.61420	.10345	.231322	.054
POST TET	5	.753	6.397	7.150	6.92000	.13413	.299932	.090
PRE TMPS	5	1.160	6.310	7.470	6.72600	.20743	.463821	.215
POST TMPS	5	.850	5.973	6.823	6.39320	.15318	.342529	.117

Table F-7. Descriptive statistics for salmonella growth in M9 supplemented with 10% sterile filtered cecal contents from 5 individual horses treated with oxytetracycline (TET), or trimethoprim-sulfamethoxazole (TMPS)

TET Treatment	Mean	Standard Deviation	95%CI	N
time 0	3.17E+03	2.37E+03	1067.069035	19
time 2	1.16E+04	4.53E+03	2805.479046	10
time 4	1.10E+05	9.11E+04	53827.87927	11
time 6	2.25E+05	1.15E+05	71131.04936	10
time 8	1.92E+06	1.02E+06	629226.4077	10
time 10	4.24E+06	1.99E+06	1231037.633	10
TMPS Treatment	**Mean**	**Standard Deviation**	**95%CI**	**N**
time 0	2.59E+03	1.44E+03	663.0500097	18
time 2	1.61E+04	5.40E+03	3349.823031	10
time 4	1.16E+06	3.61E+05	223516.9306	10
time 6	4.30E+06	2.00E+06	1241653.571	10
time 8	8.07E+06	1.28E+06	796435.4601	10
time 10	8.41E+06	1.89E+06	1169955.329	10

Table F-7. Continued

M9 ONLY	Mean	Standard Deviation	95%CI	N
time 0	2.50E+03	1605.739705	1573.595034	4
time 2	1.35E+04	707.1067812	979.9813937	2
time 4	8.85E+04	38890.87297	53898.97665	2
time 6	2.23E+05	94752.30868	131317.5068	2
time 8	1.55E+06	636396.1031	881983.2543	2
time 10	3.35E+06	2192031.022	3037942.32	2

Table F-8. Descriptive statistics for salmonella growth in M9 supplemented with 10% sterile filtered cecal contents from 5 individual horses treated with control (no treatment) or ceftiofur sodium (NAX)

CONTROL Treatment	Mean	Standard Deviation	95%CI	N
time 0	1.15E+06	581038.3426	360124.4584	10
time 2	3.09E+06	1104515.982	684573.1009	10
time 4	8.23E+06	1778295.064	1102177.773	10
time 6	8.44E+06	1557383.846	965258.1817	10
time 8	8.65E+06	1667499.792	1033507.456	10
time 10	8.74E+06	1889855.962	1171322.621	10
NAX Treatment	**Mean**	**Standard Deviation**	**95%CI**	**N**
time 0	9.51E+05	520265.9576	322458.0591	10
time 2	2.56E+06	718363.1084	445237.614	10
time 4	5.39E+06	1023555.893	634394.4705	10
time 6	5.88E+06	1054672.145	653680.1569	10
time 8	6.37E+06	1233828.729	764720.4501	10
time 10	5.84E+06	1456937.427	903002.0282	10
M9 ONLY	**Mean**	**Standard Deviation**	**95%CI**	**N**
time 0	7.25E+05	360624.4584	499790.5108	2
time 2	1.14E+06	502045.8146	695786.7895	2
time 4	3.70E+06	1131370.85	1567970.23	2
time 6	4.13E+06	1308147.545	1812965.578	2
time 8	4.55E+06	1484924.24	2057960.927	2
time 10	5.10E+06	2545584.412	3527933.017	2

Table F-9. Descriptive statistics for all salmonella growth in LB broth supplemented with 10% sterile filtered cecal contents pooled from 5 horses treated with no treatment, ceftiofur (NAX), oxytetracycline (TET), or trimethoprim-sulfamethoxazole (TMPS). Units=CFU/ml, N=number of dilutions counted.

		Mean	Standard Deviation	95%CI	N
TIME 0	CONTROL LB	7.10E+06	3535533.906	4899906.969	2
	10% NO AB	6.68E+06	3066485.502	3469989.128	3
	10% TMPS	1.10E+07	6668520.576	6535026.088	4
	10% TET	6.75E+06	3118225.991	3528537.892	3
	10% NAX	7.88E+06	3365016.097	3807801.885	3
TIME 2 h	CONTROL LB	2.92E+07	13510582.27	13240119.24	4
	10% NO AB	2.25E+07	15719494.27	15404811.9	4
	10% TMPS	1.06E+07	2157158.625	2441008.435	3
	10% TET	2.16E+07	9220403.1	9035823.481	4
	10% NAX	2.72E+07	18014901.24	17654268.02	4
TIME 4 h	CONTROL LB	9.95E+07	29881474.19	29283288.73	4
	10% NO AB	9.48E+07	30645119.24	30031646.66	4
	10% TMPS	4.74E+07	25166843.27	24663038.14	4
	10% TET	5.08E+07	13998095.11	13717872.75	4
	10% NAX	1.07E+08	28447187.91	27877714.86	4
TIME 6 h	CONTROL LB	1.36E+08	26171294.71	25647381.86	4
	10% NO AB	1.45E+08	68819976.51	67442296.49	4
	10% TMPS	8.95E+07	20327157.53	19920236.17	4
	10% TET	1.66E+08	68409861.62	67040391.53	4
	10% NAX	1.68E+08	93185728.52	91320280.11	4
TIME 8 h	CONTROL LB	1.83E+08	68024848.4	66663085.74	4
	10% NO AB	1.80E+08	74925385.11	73425483.32	4
	10% TMPS	1.54E+08	50956811.78	49936727.43	4
	10% TET	1.60E+08	41579351.85	40746991.17	4
	10% NAX	1.38E+08	48780964.53	47804437.6	4
TIME 10 h	CONTROL LB	1.68E+08	68454364.36	67084003.39	4
	10% NO AB	1.70E+08	36278460.09	35552215.88	4
	10% TMPS	2.73E+08	122904149.6	139076497.5	3
	10% TET	2.11E+08	112076424.8	155327050.9	2
	10% NAX	1.31E+08	22815984.89	22359240.67	4
TIME 14 h	CONTROL LB	3.38E+08	362794076.6	317996991.5	5
	10% NO AB	2.62E+08	97085597.97	109860610.5	3
	10% TMPS	2.16E+08	150693297.8	170522281.9	3
	10% TET	2.40E+08	1381097480	1353449834	4
	10% NAX	2.07E+08	50866844.15	49848560.82	4

Table F-10. Descriptive statistics for all salmonella growth in M9 minimal medium supplemented with 10% sterile filtered cecal contents pooled from 5 horses treated with control (no treatment) or ceftiofur sodium (NAX), oxytetracycline (TET), or trimethoprim-sulfamethoxazole (TMPS). Units for mean measurement are CFU/ml, and N=number of dilutions counted.

		Mean	Standard Deviation	95%CI	N
TIME 0	CONTROL M9	2.52E+06	2667011.436	2337694.212	5
	10% NO AB	2.11E+06	2317270.439	1716625.372	7
	10% TMPS	1.56E+06	918257.4854	680241.7495	7
	10% TET	1.79E+06	1343328.205	1074866.021	6
	10% NAX	1.74E+06	1001527.809	741928.0974	7
TIME 2 h	CONTROL M9	2.29E+06	1246025.682	1092168.929	5
	10% NO AB	2.22E+06	1084804.13	868007.6054	6
	10% TMPS	2.89E+06	1513861.508	1211318.492	6
	10% TET	2.48E+06	1295382.826	1036502.456	6
	10% NAX	3.87E+06	2294268.947	2010977.218	5
TIME 4 h	CONTROL M9	7.38E+06	4891063.279	4793151.009	4
	10% NO AB	1.51E+07	3879003.308	3801351.068	4
	10% TMPS	9.15E+06	2143983.831	2101064.263	4
	10% TET	7.70E+06	3340658.618	3273783.288	4
	10% NAX	1.17E+07	1354929.272	1327805.477	4
TIME 6 h	CONTROL M9	1.37E+07	1248999.6	1223996.368	4
	10% NO AB	1.93E+07	5068448.152	4966984.884	4
	10% TMPS	1.21E+07	822597.512	806130.2562	4
	10% TET	1.50E+07	9447927.462	9258793.122	4
	10% NAX	1.95E+07	1734694.978	1699968.802	4
TIME 8 h	CONTROL M9	1.28E+07	1326649.916	1300092.234	4
	10% NO AB	2.29E+07	1623524.972	1591024.264	4
	10% TMPS	1.59E+07	5483611.948	5373837.68	4
	10% TET	1.86E+07	2059935.274	2018698.241	4
	10% NAX	1.88E+07	2609597.67	2557357.162	4
TIME 10 h	CONTROL M9	1.49E+07	3424787.098	3356227.633	4
	10% NO AB	2.19E+07	5198317.035	5094253.973	4
	10% TMPS	1.78E+07	1159022.577	1135820.56	4
	10% TET	1.94E+07	1567109.866	1535738.511	4
	10% NAX	2.09E+07	1422146.265	1393676.879	4
TIME 12 h	CONTROL M9	1.33E+07	2578597.81	2526977.876	4
	10% NO AB	2.48E+07	2292015.125	2246132.177	4
	10% TMPS	1.73E+07	3933933.57	3855181.703	4

Table F-10. Continued

		Mean	Standard Deviation	95%CI	N
12 h	10% TET	1.79E+07	5102940.329	5000786.575	4
	10% NAX	2.23E+07	3910136.4	3831860.919	4
TIME 24 h	CONTROL M9	1.50E+07	1838477.631	2547951.624	2
	10% NO AB	2.60E+07	3394112.55	4703910.69	2
	10% TMPS	1.91E+07	565685.4249	783985.115	2
	10% TET	2.56E+07	919238.8155	1273975.812	2
	10% NAX	2.55E+07	3252691.193	4507914.411	2

Table F-11. Descriptive statistics for salmonella growth in M9 minimal medium supplemented with sodium chloride, acetate, butyrate, or propionate at 30 or 100mM

		Mean	Standard Deviation	95%CI	N	SEM
TIME 0	CONTROL 30mM NaCl	1.32E+05	2.36E+05	1.75E+05	7	89237.7
	CONTROL 100mM NaCl	1.09E+05	1.52E+05	1.21E+05	6	61906.0
	ACETATE 30mM	9.44E+04	1.31E+05	1.04E+05	6	53313.3
	ACETATE 100mM	1.01E+05	1.41E+05	1.13E+05	6	57502.2
	BUTYRATE 30mM	1.51E+05	2.54E+05	1.88E+05	7	95846.8
	BUTYRATE 100mM	1.16E+05	1.95E+05	1.45E+05	7	73829.1
	PROPRIONATE 30mM	1.29E+05	1.83E+05	1.46E+05	6	74610.5
	PROPRIONATE 100mM	1.00E+05	1.38E+05	1.10E+05	6	56194.3
TIME 2 h	CONTROL 30mM NaCl	2.42E+05	2.74E+05	2.68E+05	4	136884.6
	CONTROL 100mM NaCl	2.50E+05	3.15E+05	3.09E+05	4	157556.5
	ACETATE 30mM	3.43E+05	5.73E+05	5.61E+05	4	286262.8
	ACETATE 100mM	1.30E+05	1.80E+05	1.44E+05	6	73486.8
	BUTYRATE 30mM	2.15E+05	2.70E+05	2.00E+05	7	102175.0
	BUTYRATE 100mM	2.06E+05	3.18E+05	2.54E+05	6	129775.0
	PROPRIONATE 30mM	1.70E+05	2.46E+05	1.96E+05	6	100236.5
	PROPRIONATE 100mM	1.09E+05	1.53E+05	1.22E+05	6	62433.4
4	CONTROL 30mM NaCl	4.34E+06	2.31E+06	2.27E+06	4	1156289.9

Table F-11. Continued

		Mean	Standard Deviation	95%CI	N	SEM
	CONTROL 100mM NaCl	4.45E+06	9.51E+05	9.32E+05	4	475576.4
	ACETATE 30mM	2.22E+06	2.86E+06	2.80E+06	4	1427786.0
	ACETATE 100mM	2.17E+05	2.01E+05	1.49E+05	7	75828.4
	BUTYRATE 30mM	9.81E+05	1.12E+06	9.86E+05	5	502887.1
	BUTYRATE 100mM	1.87E+05	2.24E+05	1.47E+05	9	83928.0
	PROPRIONATE 30mM	2.41E+05	2.57E+05	1.90E+05	7	97025.2
	PROPRIONATE 100mM	1.00E+05	1.61E+05	1.05E+05	9	59758.1
TIME 6 h	CONTROL 30mM NaCl	1.41E+08	1.10E+08	1.08E+08	4	54909584.8
	CONTROL 100mM NaCl	1.12E+08	1.01E+08	9.90E+07	4	50504744.0
	ACETATE 30mM	2.27E+07	1.83E+07	1.47E+07	6	7489537.1
	ACETATE 100mM	1.22E+06	1.37E+06	1.20E+06	5	610938.6
	BUTYRATE 30mM	1.24E+07	1.74E+07	1.71E+07	4	8719381.7
	BUTYRATE 100mM	2.36E+05	3.75E+05	2.78E+05	7	141662.3
	PROPRIONATE 30mM	1.24E+06	1.34E+06	9.95E+05	7	507786.2
	PROPRIONATE 100mM	1.43E+05	2.74E+05	2.03E+05	7	103549.2
TIME 8 h	CONTROL 30mM NaCl	2.18E+08	1.05E+08	8.39E+07	6	42805503.3
	CONTROL 100mM NaCl	2.10E+08	9.21E+07	7.37E+07	6	37604742.6
	ACETATE 30mM	9.80E+07	5.49E+07	5.38E+07	4	27452990.1
	ACETATE 100mM	4.48E+06	4.82E+06	3.85E+06	6	1966734.5
	BUTYRATE 30mM	4.14E+07	2.91E+07	2.85E+07	4	14551138.3
	BUTYRATE 100mM	7.18E+05	9.06E+05	6.71E+05	7	342319.6
	PROPRIONATE 30mM	6.65E+06	6.95E+06	5.56E+06	6	2837475.9
	PROPRIONATE 100mM	2.22E+05	4.61E+05	3.42E+05	7	163793.6
TIME	CONTROL 30mM NaCl	2.11E+08	2.87E+07	2.81E+07	4	14355602.9
	CONTROL 100mM NaCl	2.10E+08	3.80E+07	3.72E+07	4	18993419.9

Table F-11. Continued

		Mean	Standard Deviation	95%CI	N	SEM
	ACETATE 30mM	2.11E+08	4.08E+07	4.00E+07	4	20422108.1
	ACETATE 100mM	1.26E+07	1.45E+07	1.27E+07	5	6469961.4
	BUTYRATE 30mM	1.08E+08	3.23E+07	3.17E+07	4	16152270.2
	BUTYRATE 100mM	1.75E+06	2.30E+06	1.70E+06	7	869133.1
	PROPRIONATE 30mM	1.64E+07	1.57E+07	1.37E+07	5	7012232.2
	PROPRIONATE 100mM	2.82E+05	5.34E+05	3.95E+05	7	201735.8

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BIOGRAPHICAL SKETCH

Tamara Shea Vetro Widenhouse was born April 15, 1970, in Fort Lauderdale, Florida, the first and only child of Ronald and Vicky Vetro. She attended the University of Florida in Gainesville, Florida, and received a Bachelor of Science degree in animal science from the College of Agriculture in 1992. She continued on toward fulfillment of a lifelong aspiration to become a veterinarian, by accepting a place in the College of Veterinary Medicine's Class of 1999, pausing briefly along the way to marry Christopher W. Widenhouse in 1995. She graduated with high honors from the University of Florida's College of Veterinary Medicine in 1999. Tamara was also accepted into graduate school and commenced work on her PhD the same year as starting veterinary school, and worked simultaneously toward both degrees. After graduation from veterinary school, Tamara was awarded the inaugural Deedie Wrigley-Hancock Fellowship in Equine Colic Research in the Department of Large Animal Clinical Sciences at the University of Florida, under the guidance of Dr. Guy D. Lester and Dr. Alfred M. Merritt—directors of the internationally renowned Island Whirl Equine Colic Research Laboratory. She also became a mother to two wonderful children during her graduate career, Alexis Mackenna in 2000 and Carissa Mackenzie in 2002, and is now expecting twins in September 2004. She moved to Pembroke Pines, Florida, in 2001 to finish work on this document, be a full-time mom, and manage Veterinary Medical Solutions Incorporated with her husband, a small biotech research and manufacturing

company focusing on veterinary product development. Her future plans include a residency in large animal internal medicine.