MICROBIAL SOURCE TRACKING: DETERMINATION OF ANIMAL SOURCES OF ENTEROCOCCI IN OSO CREEK, NUECES COUNTY, TEXAS

by

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May 2012

A Thesis Submitted
In Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE IN BIOLOGY

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Format: *Journal of Applied Microbiology*

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ABSTRACT

Enterococci are a genus level group of gram-positive facultative anaerobes found primarily in the intestinal tract of mammals and birds. This characteristic, and their role as fecal indicator bacteria for water quality standards, makes them ideal for use in microbial source tracking (MST) studies. An MST study conducted in a south Texas coastal watershed, involved the creation of a library of carbon source utilization (CSU) and antibiotic resistance profiles (ARP) of enterococci from multiple animal hosts. A total of 1,369 enterococci were isolated from sewage, livestock, domestic, and wild animal fecal samples for the library, and 824 isolates were filtered from creek water and sediments via EPA Method 1600. The MicroLog™ (Biolog Inc.) Microbial Identification System (MIS) was used to create CSU profiles, while ARPs were created using 21 antibiotics through the Kirby-Bauer disk diffusion method and the Biomic™ Microbiology Analyzer. Profiles of animal and creek isolates were compared using two statistical techniques - linear discriminant analysis (LDA) and random forests (RF). Speciation using the MicroLog™ MIS showed certain animals harbored fewer species than others and some *Enterococcus* spp. were only associated with particular hosts. Combining CSU profiles and ARPs in a toolbox approach, allowed for source identification of creek and sediment enterococci. Both avian (inland species) and non-avian wildlife were found to be responsible for the majority of contamination within the creek using LDA and RF analysis. Models developed using LDA outperformed RF when using both CSU profiles and ARPs. This study demonstrated that using multiple laboratory and

statistical methods in a “toolbox” approach, could both characterize and identify animal fecal sources of enterococci from within the environment. These results provide valuable source information for use in developing remediation plans to reduce the levels of contamination in a rural coastal watersheds.

ACKNOWLEDGMENTS

This research project represents the culmination of over 11 years of graduate student microbial research into indicators, markers, and sources of contamination within the Oso watershed. Sara Heilman, Katie Crysup, Zeke Campbell, Marc Carpenter, Michelle Lindsey, and myself have all labored towards the common goal of microbiologically understanding the Oso watershed within Nueces County, Texas. We have followed the lead of several diligent environmental investigators such as: Joanna Mott, Richard Hay, Roy Lehman, and Brien Nicolau. Their examples have ultimately led us, the students, to utilize science as a tool to discover, examine, and identify microbial contamination within Oso Creek. The conclusion of this project is as much their accomplishment, as is it is mine. Our hope is that one day, this waterway, that hosts numerous human developments as well as both animal and plant habitat, may yet be reclaimed, and that our research may further lead to understanding and development of global water quality.

The Texas State Soil and Water Conservation Board along with the Coastal Bend Bays and Estuaries Program funded this project, with facilities provided by Texas A&M University – Corpus Christi. This project would not have been possible without the mentoring and support of my committee members: Drs. Joanna Mott, Gregory Buck, Roy Lehman, and Blair Sterba-Boatwright. Each one of you cultivated my understanding of science and research, and I am extremely grateful for your stewardships, thank you. Special appreciation goes to the United States Environmental Protection Agency, Texas Parks and Wildlife,

Center for Water Supply Studies, United States Department of Agriculture, Texas Commission on Environmental Quality, City of Robstown, Corpus Christi International Airport, participating farmers, ranchers, and veterinary establishments of Nueces County, and the Texas AgriLife Research and Extension Center at Corpus Christi.

My deepest gratitude goes to those who went out of their way to ensure the fruition of this project, specifically: Richard Hay, Dr. John Young, Sandra Favela, Dr. Juan Landivar, Kenneth Schaefer, Joshua Haslun, and Randall Landry. Special thanks also goes to members of the Texas A&M University – Corpus Christi Environmental Microbiology Lab both past and present, specifically: LaDonna Henson, Cody Stewart, Lijie “Summer” Shi, Miguel Diaz, Amanda Smith, Louise Lyle, Alyson Doyungan, Leslie Strong, Samantha Hune, Michael Birchfield, Marc Carpenter, Russel Grandbois, Shane McIntyre, Dr. Guadalupe Vidal, Kasey Disher, Hoang “Kim” Pham, Shawntey Fogel, Elizabeth Hager, Eliud Aleman, and Christine Bowen.

I would also like to thank my family for their support, especially my Aunt and Uncle, Millie and Scott Williamson for their never-ending hospitality, warm meals, and for welcoming me into their home like a son. Lastly, I would like to thank above all, the two people that have devoted their lives to my cause and education, my parents, Sarah and Enrique Rodriguez. Their unyielding love and support have sustained me through countless challenges and many late nights. Thank you Mom and Dad, this is both for you and only possible because of you.

INTRODUCTION

Fecal contamination of waterways is an endemic problem prevalent in all nations around the world regardless of socio-economic status. This problem results in closed beaches, tainted shellfish beds, and polluted waterways, which can negatively impact public health and result in economic losses. Globally, the World Health Organization estimates that 3.4 million fatalities each year can be attributed to water-related diseases stemming from contaminated water-bodies (Dufour *et al*. 2003). In the United States alone, water pollution and waterborne outbreaks have cost millions of dollars in direct and indirect costs, as well as numerous human lives (US EPA 2005; Stewart *et al*. 2007).

From 1986-2006, the United States Centers for Disease Control and Prevention (US CDC) and United States Environmental Protection Agency (US EPA) documented 6,682 laboratory confirmed cases of illness due to bacterial, viral, or protozoan outbreaks in recreational U.S. freshwaters (US EPA 2003; US CDC 2004, 2006, 2008). One of the most severe water contamination outbreaks in U.S. history occurred in 1993 in Milwaukee, Wisconsin, when the protozoan, *Cryptosporidium parvum,* caused an estimated 400,000 cases of gastroenteritis that were attributed to possible fecal contamination from human or livestock in a water-body. The outbreak resulted in the loss of more than 100 lives and over 90 million dollars of direct medical costs and productivity losses (US CDC 2001; Corso *et al*. 2003; Dawson 2003; Zhou *et al*. 2003).

Aside from direct public health concerns and medical costs, contaminated waters in the U.S. can inflict economic damage on the tourism industry. In 2010

alone, U.S. beach closings and advisories were issued for over 24,000 days with 70% of these closures and advisories attributed to elevated levels of fecal bacteria (Dorfman and Rosselot 2011). Lake Michigan beach closures were estimated to cost the local tourism industry over $37,000 a day and a typical "swimming day" for the nation's beaches has been valued at about $35.00 per visiting individual (Rabinovici *et al*. 2004; Kildow *et al*. 2009). Approximately 85% of all U.S. tourism revenue is generated from U.S. coastal states and shoreline counties, with a 2007 estimate noting that these areas contribute 5.7 trillion dollars of the U.S. gross domestic product alone (Kildow *et al*. 2009).

Fecal contamination of shellfish harvesting waters can also create economic and public health concerns. Mollusks such as oysters, clams, mussels, and scallops are valuable commodities both as food sources and as sources of industry for coastal areas. The U.S. shellfish industry commercially accounted for nearly 50% of the value of overall commercial fishery from 1984 to 1993 (National Marine Fisheries Service 1996; Meschke and Boyle 2007) and a U.S. survey of commercial shellfish calculated an estimated value of 200 million dollars for 1995 (Alexander 1998; Meschke and Boyle 2007). Shellfish are filter feeders, which remove particulate matter from their surrounding waters and utilize it as a food source. Consequently, they can internally bioaccumulate microorganisms to much higher concentrations than those found in ambient waters (Bouchriti and Goyal 1993; Geary and Davies 2003; Meschke and Boyle 2007). Levels of pathogens have been found to be as much as 100 times higher inside shellfish as opposed to levels of pathogens in the water column, posing a

risk for human consumption (Rippey 1994; Wilson and Moore 1996; Morris 2003). Enteric pathogens such as *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., *Escherichia coli (E. coli),* Hepatitis A, and norovirus, have been shown to account for 80% of reported disease outbreaks attributed to shellfish (Wittman and Flick 1995; Potasman *et al*. 2002; Meschke and Boyle 2007). The number of U.S. food-borne illnesses is estimated at 76 million cases of illness per year and shellfish are estimated to be responsible for 4.5 million of those cases annually (Mead *et al*. 1999; Wallace *et al*. 1999; Meschke and Boyle 2007).

In order to protect the nation's waters, economy, and its people, the United States Congress passed the Federal Water Pollution Control Act of 1948 (33 U.S.C. 1251-1376 P.L. 80-845), creating a legislative basis and funding measure for the control of water pollution. The act was subsequently amended many times, in 1956, 1961, 1965, 1966, 1970, 1972, 1977, 1981, 1987, and 2002 (US EPA 2004b, 2011b). The amendment of 1972 was the most notable, as it significantly expanded and reorganized the original act of 1948. The amendment of 1972 was popularized as the "Clean Water Act" (US EPA 2011b). The Clean Water Act and its amendments have set forth a broad statutory framework for regulation in order to "restore and maintain the chemical, physical, and biological integrity of the Nation's waters" (US 33rd Congress 2002). The Clean Water Act implemented pollution control programs, wastewater standards for municipalities and industrial wastes, and set water quality standards for surface waters throughout the nation. In setting these standards, it mandates states to monitor their water bodies for chemical, physical, and biological contamination. Water

bodies that fail to meet designated standards are classified as impaired and placed on the state's Water Quality Inventory and 303(d) List for remediation. Biennially, each state submits their 303(d) List to the US EPA for update and review (US EPA 2011b). The 303(d) List is a priority ranking for surface waters that takes into account the type and severity of the pollution as well as the uses or classifications for surface waters (US 33rd Congress 2002).

Currently the leading impairment of waterways in the United States is attributed to pathogenic microorganisms (US EPA 2011a). Fecal matter can be a major source of these pathogenic microorganisms and can be derived from both human and nonhuman sources (Ferguson *et al*. 2003; Tallon *et al*. 2005; Stewart *et al*. 2007). Elevated levels of pathogens, specifically from human and animal fecal matter, poses a hazard to recreational contact and increases public health risks within water bodies (US EPA 2009a, 2009b).

The US EPA has estimated that as many as 3.5 million people are sickened each year because of contact with human fecal material (Dorfman and Rosselot 2011). Exposure to fecally polluted water bodies can cause illness due to pathogenic bacteria, protozoa, and viruses. Pathogens common to fecally polluted waters bodies include: *E. coli, Salmonella* spp., *Campylobacter* spp., *Shigella* spp., *Klebsiella* spp., *Entamoeba* spp., *Naelgeria* spp., *Cryptosporidium* spp., *Giardia* spp., Adenovirus, Hepatitis A virus, and Enteroviruses (Stewart *et al*. 2007). Signs, symptoms, and illnesses associated with these pathogens include: abdominal cramps, diarrhea, fever, vomiting, and ear, skin, and respiratory infections (US EPA 2009a). Bacterial infectious doses vary from 10^1

to 10^8 bacteria while viral and protozoan infectious doses are often much smaller with ranges between 1 to 100 infective particles (virons, protozoan, cyst, or oocyst) (Yates and Gerba 1998; US EPA 2004b). Recreational water exposure results in illness when pathogens from contaminants suspended in the water column enter the body. These pathogens can be ingested orally or enter through other mucosa such as eyes, ears, nose, anus, genitourinary tract, or dermal abrasion (Henrickson *et al*. 2001; US EPA 2004b).

As many as 400 different species of bacteria are found in the intestines of mammals alone, with bacterial densities as high as 10^{10} to 10^{11} per gram concentration in fecal matter (Zoetendal *et al*. 2004). Enteric viruses can number from 10^3 to 10^{12} and protozoan parasites can number as high as 10^6 to 10^7 per gram of feces in an infected individual (US EPA 2004b). Since it is not yet practical to identify and enumerate the full spectrum of fecal pathogens due to cost limitations and low detection rates, fecal indicators have been used to assay water quality. Ideally, fecal indicators should be easily detected and enumerated, be nonpathogenic, have similar survivability in both fresh and marine waters, and concentrations should be strongly correlated with levels of pathogens and swimming related illness (Scott *et al*. 2002; Noble *et al*. 2003; Wade *et al*. 2003). Fecal coliforms, *E. coli*, and *Enterococcus* have all been used as fecal indicator bacteria to assay microbial contamination of waterways due to their correlation with fecal pollution and risk of disease (US EPA 1976, 1986; Scott *et al*. 2002; Noble *et al*. 2003; Wade *et al*. 2003, 2006, 2008, 2010).

Coliform bacteria were typically employed as microbial indicators to assess drinking water quality and safety for the majority of the 20th century (Leclerc *et al*. 2001; Stewart *et al*. 2007). Coliforms are aerobic and facultative anaerobic, gram-negative, non-spore forming, rod-shaped bacteria, with the family *Enterobacteriaceae* containing many members of the coliform bacteria group. Select members of coliforms can be pathogenic towards humans and are thought to originate in the intestines of warm-blooded animals (Carrero-Colón *et al*. 2011). Coliforms that can ferment lactose with gas formation while incubated at 35 ^{o}C after 48 hours are termed "total coliforms," and members of total coliforms include *E. coli, Enterobacter* spp., *Klebsiella* spp., and *Citrobacter* spp. (Carrero-Colón *et al*. 2011). The term "fecal coliform" has been given to thermotolerant members of the total coliform group that are further able to ferment lactose at 44.5 ^{o}C (Stewart *et al*. 2007). The subgroup of fecal coliforms was established in an effort to detect organisms exclusively from fecal origins and fecal coliform bacteria have been shown to be present in concentrations as high as 10^8 to 10^9 organisms per gram of feces (US EPA 2004b). However, coliform bacteria, fecal coliforms included, may survive and replicate in waters and soils under certain environmental conditions outside of those found in warm-blooded intestinal tracts (Rivera *et al*. 1988; Griffin *et al*. 2001; Ishii *et al*. 2006).

Currently, meta-analysis of data from US EPA and other sources, by Wade *et al*. (2003), supports the move away from utilizing fecal coliforms as microbial indicators for fecal contamination; since there has been inconclusive evidence linking the risk of illness to increased levels of fecal coliforms (US EPA

2002a; Wade *et al*. 2003). Nevertheless, fecal coliforms are still used to assay certain water bodies and are currently recommended by the United States Food and Drug Administration (US FDA), to monitor the bacterial quality of shellfish harvesting waters (US FDA 2005).

Due to the findings that coliforms are not feces specific (Rivera *et al*. 1988), and the need for single markers of fecal indicators, a common fecal coliform that resides in the lower intestinal tract of warm-blooded animals has been used as an alternative indicator. *E. coli* are gram-negative, rod-shaped, and facultative anaerobes belonging to the family *Enterobacteriaceae,* which reside in the intestinal tract of humans and warm-blooded animals. *E. coli* are usually benign to the host intestinal lumen and are found widely distributed throughout the gut at different densities. *E. coli* are an essential part of the intestinal flora and aid in maintaining the gut physiology of its hosts (Nataro and Kaper 1998; US FDA 2011). Though most types of *E. coli* are either opportunistic pathogens or non-pathogenic, there are serogroups that are virulent. Notably, *E. coli* O157:H7 contains the Shiga toxin, or verocytotoxin, and causes enterohemorrhagic disease. Other serogroups containing this virulence mechanism include O26, O111, and O103. Several other serogroups of *E. coli,* however, are also classified as pathogenic such as enterotoxigenic, enteropathogenic, enteroinvasive, and enteroaggregative *E. coli* (Levene 1987; Nataro and Kaper 1998).

E. coli are shed in significant numbers per gram of feces of warm-blood animals but environmental reservoirs have been identified (Rivera *et al*. 1988;

Solo-Gabriele *et al*. 2000; Byappanahalli and Fujioka 2004; Ishii *et al*. 2006; Meschke and Boyle 2007). *E. coli* do not persist in marine environments and their numbers diminish quickly with time. *E. coli* has also been shown to be susceptible to certain physical conditions such as sampling locations, weather conditions, and temperature fluctuations (Fujioka and Yoneyama 2002; Noble *et al*. 2003; Meschke and Boyle 2007).

Currently, the US EPA recommends *E. coli* or *Enterococcus* as fecal indicator bacteria for freshwaters and only recommends the use of *Enterococcus* for marine waters (US EPA 1986). Epidemiological studies on recreational water use and incidences of gastroenteritis have established a significant association between *E. coli* concentrations and gastroenteritis in fresh water, and *Enterococcus* concentrations with gastroenteritis in marine water, supporting current US EPA recommendations (US EPA 1984; Wade *et al*. 2003, 2006, 2008, 2010).

Enterococci are gram-positive, non-spore forming, chemoorganotrophic, lactic acid bacteria that are cocci in shape and occur in diploid formations or single chains. Taxonomically, enterococci belong to the phylum/division *Firmicutes*, class *Bacilli*, order *Lactobacillales*, and family of *Enterococcaceae* (Carrero-Colón *et al*. 2011). These bacteria are normally found as members of mammalian and avian intestinal flora but can also be associated with plants, insects, and algae. They have been used since the early 1900's as indicators of fecal contamination in water (Jouhaud 1903; Andrewes and Horder 1960; Wessels *et al*. 1990; Müller *et al*. 2001; Whitman *et al*. 2003; Fisher and Phillips

2009). There are approximately 40 recognized enterococcal species; with species such as *Enterococcus faecalis, E. faecium, E. durans,* and *E. hirae* principally associated with feces (Godfree *et al*. 1997; Carrero-Colón *et al*. 2011). *E. faecalis* colonizes the large intestines in humans and animals, and approximately 10^5 to 10^7 organisms are shed per gram of feces, while *E. faecium* is shed at a concentration of 10^4 to 10^5 organisms per gram of feces in humans (Meschke and Boyle 2007; Fisher and Phillips 2009). Overall, enterococci levels in humans reach 10^8 colony forming units per gram of feces but typically only represent 1% of human intestinal flora (Tendolkar *et al.* 2003).

Enterococci are facultative anaerobes classified by their ability to grow in the presence of azide and 6.5% sodium chloride broth at pH 9.6, at 10 °C and at 45 °C, with resistance to 60 °C for 30 min, and by their ability to reduce 0.1% methylene blue (APHA 2005; Devriese *et al*. 2006; Stewart *et al*. 2007). Enterococci can hydrolyze esculin in the presence of 40% bile salts and most species are non-motile. The genus lacks cytochrome enzymes, and for this reason most enterococci are catalase negative, though some do possess pseudocatalase activity (Devriese *et al*. 2006). They are able to ferment a variety of carbohydrates, including D-glucose, D-fructose, D-mannose, ribose, galactose, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, and β-gentiobiose, which can aid in phenotyping the bacteria (Huycke 2002; Devriese *et al*. 2006). Enterococci have been utilized in food processing, for example cheese maturation, and as probiotics for animal feeds due to their ability to break down milk-associated sugars and in some, the ability to produce

antimicrobial compounds (Bennik *et al*. 1998; Morandi *et al*. 2005; Weiss *et al*. 2005). Prior to 1984, enterococci were categorized as Lancefield Group D fecal streptococci due to the glycerol teichoic acid D-antigen within their cell walls (Carrero-Colón *et al*. 2011). Certain species of *Enterococcus* can be opportunistic and nosocomial pathogens, with *E. faecalis* and *E. faecium* frequently isolated from nosocomial enterococcal infections (Franz *et al*. 1999; Facklam *et al*. 2002; Fisher and Phillips 2009). Contributing to the pathogenicity of *Enterococcus* is the ability of the bacteria to acquire antibiotic resistance, especially to aminoglycosides and vancomycin (Bonten *et al*. 2001; Donabedian *et al*. 2003).

Since enterococcal levels have been shown to have a strong correlation to fecal pollution and swimming-associated gastrointestinal illnesses, and that enterococci are primarily found in the lower intestinal tract and feces of mammals and birds, its presence in impaired waterways is indicative of fecal contamination. Enterococci have also been shown to be more resistant to environmental stressors such as wide temperature ranges, high light levels, increased salinity, and low turbidity, than fecal coliforms or *E. coli* (Rees 1993; Alkan *et al*. 1995; Cools *et al*. 2001; Kay *et al*. 2005).

Fecal indicators can be used to assess levels of contamination; however, their presence or concentration does not provide information about potential sources of contamination, which is needed to conduct accurate risk assessments, choose effective remediation strategies, and bring polluted waters into compliance with regulatory policies. The concept of tracing bacteria to the

origins of fecal contamination has been termed microbial source tracking (MST) (Meays *et al*. 2004), and several methods have emerged to discriminate among different fecal pollutant sources. There is currently no single method or panacea that has been accepted as a universal technique for all types of fecally contaminated water bodies, because several factors influence the level of complexity of a particular water-body and each method has its limitations. A variety of bacteriological, virological, and chemical MST tools have been developed and used, with varying degrees of discrimination, to discern sources of fecal contamination. Each of these tools varies differently in sensitivity, cost, ease of use, and methodology.

Microbial source tracking can be subdivided into two major methodologies: library dependent and library independent methods (Stoeckel and Harwood 2007). These methodologies are further divided into observable physical or biological characteristics, known as phenotypic characteristics, and genetic or DNA based characteristics, known as genotypic characteristics. Microbial source tracking methods may also be cultivation dependent, requiring growth and isolation of the target microorganism, or cultivation independent, which allows for the detection of the microorganism regardless of isolation or growth (US EPA 2005; Stoeckel and Harwood 2007).

Library dependent MST methods involve the collection of bacterial isolates from various animal sources to form a reference library that can be used to identify sources of isolates collected from a contaminated environment. Library dependent methods rely on profiling unknown source bacterial strains isolated

from impaired bodies of water and comparing these profiles with the bacterial profiles in the library from various hosts (*e.g.*, humans, cattle, swine, wildlife, and avian) and environmental sources (*e.g.*, municipal wastewater, agricultural runoff) (US EPA 2005). These profiles, or "fingerprints", serve as unique identification patterns and are distinct for animal sources (Simpson *et al*. 2002). Library dependent methods include antibiotic resistance analysis, carbon source utilization profiling, ribotyping, pulse field gel electrophoresis (PFGE), amplified fragment length polymorphism, and repetitive element sequence-based PCR (rep-PCR) (US EPA 2005; Stoeckel and Harwood 2007).

Library independent methods do not require the construction of a reference library but instead detect matches to identifiers, genetic markers or genetic profiles. These methods include coliphage typing using viruses that are specific to the intestinal tract, chemical approaches using fecal sterols and ammonia nitrogen (NH_3-N) levels, as well as gene specific PCR and total community analysis via 16S ribosomal RNA (Sinton 1998; US EPA 2005). Examples of host-specific markers are the human-specific and ruminant-specific *Bacteroides* 16S rRNA markers, markers for the human-specific Archaean *Methanobrevibacter smithii*, and Human Polyoma Viruses, the human-specific enterococcal surface protein (*esp)* gene, and ruminant-specific toxin markers from strains of enterotoxigenic *E. coli* (Bernhard and Field 2000; Scott *et al*. 2005; Seurinck *et al*. 2005; Ufnar *et al*. 2005; US EPA 2005; McQuaig *et al*. 2006).

Library independent and genetic methods have been utilized in MST studies for highly reproducible, automated, and rapid results. However, there are concerns regarding geographical stability of genetic markers, specificity and sensitivity, interpretation of results in relation to regulatory water quality standards, and high start-up costs associated with many genetic methods (US EPA 2005; Casarez *et al*. 2007; Stoeckel and Harwood 2007; Mott and Smith 2011). Utilizing phenotypic methods has been a reliable way to identify contamination in specific geographic areas and has been utilized successfully by numerous researchers since the mid-1990s (Mott and Smith 2011). Phenotypic methods that rely on source libraries include a variety of different phenotypic tests that can profile source isolates with minimal training of personnel, low start-up costs, and standardized methods (US EPA 2005; Mott and Smith 2011).

Creation of a phenotypic library involves surveying the potential sources of the fecal bacteria present in the watershed, selecting appropriate phenotypic assays, and calculating the number of isolates needed to create a statistically valid representation of the watershed's fecal bacteria population (US EPA 2005). In order to avoid underrepresentation, highly confined geographic regions are recommended with multiple host and environmental samples (McLellan 2004; Mott and Smith 2011). No model or defined sample size has been established for MST, but many phenotypic-based source tracking studies have utilized large known-source libraries consisting of 1,000 - 6,000 bacterial isolates (Johnson *et al*. 2004; US EPA 2005).

Previous MST studies have shown an array of host and environmental sources that can act as major contributors of fecal contamination in waterways. Environmental point sources such as wastewater treatment plants, sewage overflows, and failing septic systems can contribute to anthropogenic bacterial pollution and can drastically reduce water quality (Simpson *et al*. 2002). Human fecal material has been implicated as a source of contamination in several MST studies. Two phenotypic-based studies in South Carolina and Florida identified sewage as a source of contamination along rural creeks (Whitlock *et al*. 2002; Kelsey *et al*. 2003). Human fecal material is relevant to public health due to the assumption that human fecal material potentially poses a greater human health risk than other types of fecal material (Sinton *et al*. 1998; Scott *et al*. 2002; Harwood 2007). However, the US EPA advises that non-human fecal matter can still pose a risk to human health based on studies linking human pathogens in warm-blooded animal feces (US EPA 2003, 2004a, 2004b, 2009b). Discrimination between human and nonhuman sources of fecal pollution can also be useful for both remediation efforts and public health aspects.

Non-point sources of fecal contamination can play a significant role in degrading water quality and are often more difficult to remediate than point sources (Simpson *et al*. 2002; US EPA 2005). Non-point sources of fecal contamination include agricultural runoff, as well as livestock and wildlife fecal pollution within the watershed, posing a risk to public health due to zoonotic pathogens that animals can harbor (Anderson *et al*. 1997; Stewart *et al*. 2007). Most bacterial, viral, and protozoan human pathogens associated with

waterborne outbreaks are common in feces of higher mammals and avian species (Leclerc *et al.* 2002; Stewart *et al*. 2007; US EPA 2009b). Zoonotic vectors can impact microbial quality and it is therefore important to identify animal sources of pollution. Livestock, specifically cattle, have been implicated as the primary contributor to fecal bacterial contamination in many waterways (Edwards *et al*. 2000; Graves *et al*. 2002; Booth *et al*. 2003; Graves *et al*. 2007). Other non-point sources such as wildlife (avian, deer, raccoon, or feral hog) can also be major contributors to bacterial pollution (Whitlock *et al*. 2002; Somarelli *et al*. 2007; Vogel *et al*. 2007). Birds, in particular, have been identified as sources of bacterial pollution where avian populations are abundant, such as shorelines and marsh areas that are home to different species of seabirds (Choi *et al*. 2003). Migratory birds can also impact water quality since increased bird populations can be present in the environment during migratory seasons (Graves *et al*. 2007; Smith *et al*. 2010).

Creating a phenotypic bacterial library from different hosts and environmental sources involves selecting specific phenotypic assays. Utilizing one or more phenotypic tests or combining data sets in a “toolbox” approach can be used to further refine the process of identifying fecal contamination by improving confidence in MST identifications (McLellan 2004; Genther *et al*. 2005; US EPA 2005; Casarez *et al.* 2007; TCEQ and TSSWCB 2007; Moussa and Massengale 2008). Among the array of phenotypic tests available, speciation via carbon source utilization (CSU) and antibiotic resistance profiling (ARP) via

Kirby-Bauer disk diffusion method remain rapid and easy to perform tests requiring limited training for personnel and low start-up costs.

Carbon source utilization (CSU) can be used to form bacterial profiles based on metabolic reactions or by-products (Kuhn *et al*. 1995; Manero *et al*. 2002; Hagedorn *et al*. 2003; Stewart 2005; Graves and Weaver 2009). The commercial MicroLog™ Microbial Identification System (Biolog, Inc., Hayward, CA) is a tool used by MST researchers to perform CSU profiling, also referred to as CUP (carbon utilization profiles), and the system can also identify bacteria, potentially to the species level by comparing profiles to a commercially available database. MicroLog™ has been evaluated by numerous researchers focusing on medical microbiology, microbial source tracking, and bioremediation and has been shown to have a high degree of accuracy in regards to identifying bacteria and speciation of *Enterococcus* (Miller and Rhoden 1991; Holmes *et al*. 1994; Hagedorn *et al*. 2003; Moore *et al*. 2006).

The Biolog Inc. system consists of a 96-well MicroPlate™ (1014; Biolog, Inc., Hayward, CA) that contains 95 discrete tests and one well that contains a control blank or water. Each of the 95 wells contains a different carbon source and an oxidized form of a color-changing reagent called tetrazolium violet. After inoculation of the 96-well MicroPlate™, a series of oxidation-reduction reactions takes place. During the bacterial respiratory process, electrons are exchanged, leading to the reduction of the tetrazolium dye present in each of the wells (Biolog 1999, 2001, 2004). The dye reacts to metabolic processes rather than metabolic by-products and the intensity of the color change is associated with the

efficacy of the reaction. The intensity of the dye can be colorimetrically measured in each well by the MicroLog™ Microbial Identification System (Biolog, Inc., Hayward, CA) to create a metabolic fingerprint of the inoculated bacteria. This fingerprint can then be compared to the Biolog commercial bacterial library in order to identify the genus and species of the bacteria.

Another phenotypic method that has been widely used in MST is antibiotic resistance profiling (ARP) (US EPA 2005; Jiang *et al*. 2007; Olivas and Faulkner 2008). Originally proposed by Wiggins (1996) for use in microbial source tracking, ARP has been successfully used to classify isolates into source categories (Wiggins 1996; Wiggins *et al*. 1999; Wiggins *et al*. 2003). This library dependent method relies on the basis that both humans and animals (domestic and wild) are exposed to an array of antibiotics via clinical or environmental influences. Over time, the frequency of exposure and concentrations of antibiotics can lead to selective pressure mechanisms, enabling antibiotic resistant flora to survive within various hosts. When exposed to antibiotics, resistance or susceptibility can be documented and used to create a characteristic profile for strains from various host and environmental sources (Choi *et al*. 2003).

There are several techniques to determine ARPs of bacteria. One method involves growing multiple isolates in microtiter plates. Each isolate is then transferred to agar plates that contain different types and concentrations of antibiotics. Antibiotic resistance is measured as growth on the plate and susceptibility or resistance profiles are created for each isolate based on the

binary observation of growth or lack of growth (Simpson *et al*. 2002). Other techniques involve the use of multi-well plates similar to the 96-well MicroPlates™ used in the Biolog system, with the exception that instead of carbon sources, each well contains a different type or concentration of antibiotic. Resistance is assayed based on the highest concentration of antibiotic in which the isolate can grow; optical densities can also be assayed and used for discriminatory capabilities (Parveen *et al*. 1997; Webster 2004).

Antibiotic resistance profiles can also be generated using the standardized Kirby-Bauer disk diffusion method (Bauer *et al*. 1966; Clinical and Laboratory Standards Institute 2006a, 2006b, 2008). This method entails inoculating known concentrations of bacteria onto Mueller Hinton agar plates. Filter paper disks with standard concentrations of antibiotics are placed on the inoculated plates. After incubation, zones of inhibition surrounding the disks are measured either by hand or, to more precisely measure zone diameters, increase reproducibility, and to reduce analyst error, automated plate reading systems can be employed such as the Biomic™ Vision Microbiology Analyzer (Giles Scientific Inc., Santa Barbara, CA). The diameters of the zones of inhibition indicate the sensitivity of the bacteria to the antibiotic and serve as the isolate's antibiotic resistance fingerprint.

Profiles of unknown source isolates can be compared to library profiles from known animal sources using various statistical methods, such as discriminant analysis and Random Forests to categorize unknown source isolates into animal source groups. Discriminant analysis (DA), which includes

linear discriminant analysis (LDA) and quadratic discriminant analysis (QDA), is one of the most widely published statistical techniques used for classifying unknown isolates using ARP and CSU methods (Hagedorn *et al*. 1999; Harwood *et al*. 2000; Ritter *et al*. 2003; Wiggins *et al*. 2003; Booth *et al*. 2005; US EPA 2005; Graves *et al*. 2007; Moussa and Massgenale 2008; Smith *et al*. 2010). Discriminant analysis (DA) is a statistical technique based on principal component analysis, which reduces and identifies dependence patterns among variables. It uses the interdependence between original variables, and via correlation or covariance, reduces the dataset to a smaller set of variables called principal components. These principal components retain most of the variation in the original dataset (Johnson and Wichern 2002; US EPA 2005).

Discriminant analysis utilizes these principal components to reduce the dimensionality of the data and then categorizes the data points into groups by algorithmically selecting a linear threshold to differentiate among classes (Huberty 1994; Johnson and Wichern 2002). Statistical assumptions for DA are that the training dataset (the known-source animal library) is derived from a random sample of a given population, and the variation between samples is described by a normal distribution, *i.e.,* a multivariate normal distribution and should be independent from one another. The population covariance matrices for the predictor variables in each group must also be equal, thus assuring homogeneity of covariance or homoscedasticity (Stevens 1992; US EPA 2005; Mertler and Vannatta 2010). In the context of MST, discriminant analysis uses estimates of covariance matrices that tend to be poor unless large sample sizes

are analyzed. The US EPA cautions the use of LDA on MST data but highlights QDA as a "somewhat reasonable approach" for phenotypic profiling data with large sample sizes (US EPA 2005).

A key issue associated with MST is the level and consequences of misclassification. Evaluation of classification error is necessary to determine potential costs of remedying insignificant sources of contamination (US EPA 2005). Once MST data have been statistically analyzed, they can be submitted to, and used by, policy makers to make decisions about contaminated water-bodies and how to implement best-management practices for remediation. In order to make effective decisions, policy makers need the most accurate assessment of the sources of the contaminant(s). Misclassification of sources can result in misdirected and costly remediation; for example, unnecessary wastewater facility upgrades, imposed best-management practices for livestock waste management, or wildlife management plans. Though discriminant analysis is the most widely used statistical tool for ARP and CSU analysis, other statistical methods have emerged that may improve classification results and minimize misclassification errors.

Random Forests (RF) is a statistical method that utilizes classification trees. Classification is based on following branches of a tree that are determined by characteristics or measurements of the object. Each tree is composed of multiple nodes that branch further to other nodes. The branches lead to different classifications of data based on characteristics determined by each node (Duda *et al*. 2000). Random Forests is based on a collection of many different

classification trees, with each tree casting a vote for the class of the object (Breiman 2001). The classification of the majority votes from all trees in the "forest" with the number of trees can vary depending on the specifications of the user. Utilizing large numbers of trees in conjunction with how they are constructed can minimize overfitting the data that can often occur when using DA.

An MST study conducted in Brazoria County, Texas on the Cow Trap and Cedar saltwater lakes, examined the use of Random Forests as a novel statistical technique on phenotypic *E. coli* ARP data. Comparisons were then made between results using LDA versus RF for data analysis. The average rates of correct classification (ARCCs) for the study's library were up to 12% higher and rates of correct classification for individual sources were up to 23% higher using RF as opposed to DA. Additionally, RF outperformed DA in comparison of training and test sets in 999 out of 1000 times (Smith 2009; Smith *et al*. 2010). This study was the first MST study to utilize RF for MST; it demonstrated a significantly higher classification rate, and therefore the potential to decrease misclassification rates in comparison with using DA for data analysis.

Situated in the Coastal Bend of south Texas, Nueces County is a coastal area of semi-arid land that hosts or contains portions of five Texas watersheds along with several estuarine and bay systems. Among these water bodies reside several impacted or impaired waterways (TCEQ 2010; EPA 2011a). The county has a human population of 340,223 (US Census 2010) out of a total metropolitan area population of over 500,000 , with the local tourism industry of the Coastal

Bend supporting around 13,000 jobs and bringing in 1.1 billion dollars annually into the local economy (Coastal Bend Bays and Estuaries Program 2010). Since 2002, Oso Creek, a waterway situated within Nueces County, has been listed on the Texas Water Quality Inventory and 303(d) List due to elevated levels of bacteria (TCEQ 2002, 2010).

In compliance with the Clean Water Act, the Texas Commission on Environmental Quality (TCEQ) has developed a Total Maximum Daily Load (TMDL) model for the creek; however, this model does not identify the source, or sources, of the bacterial contamination in the upper sections of the creek (Hay and Mott 2008). A TMDL estimates the maximum amount of contamination, such as bacterial contamination, that a water body can sustain but does not identify sources of contamination, merely that the contamination is present. Oso Creek is effluent driven, receiving permitted discharge water from the Robstown Waste Water Treatment Facility (RWWTF) (SIC Code 4952), and runs approximately 29.5 miles (47.2 km) through rural agricultural fields, cow and horse pastures, wildlife habitats, and residential developments, before discharging into Oso Bay (Nicolau 2001; TCEQ 2005). During and following rainfall, the creek can receive agricultural and urban non-point source runoff and other point source inflows such as storm water ditch discharges. Previous studies have shown that fecal bacterial (enterococci) loading occurs along the entire length of the creek during both wet and dry periods (Crysup 2002; Campbell 2004; Hay and Mott 2005). However, the source or sources of this bacterial loading during dry weather are largely unknown.

Oso Creek originates as an effluent-based and drainage freshwater creek but includes a downstream marine tidal portion, ultimately exchanging water with the estuarine system of Oso Bay. Based on the recommendation of the US EPA for freshwater and marine water bodies, as well as Oso Watershed modeling studies, *Enterococcus* has been selected as the fecal indicator to evaluate water quality throughout the entirety of Oso Creek (US EPA 1986; Crysup 2002; Campbell 2007; Hay and Mott 2007; Hay and Mott 2008).

In this MST study, known-source enterococci isolates were collected within the Oso watershed and profiled to construct a phenotypic enterococcal library via a laboratory and statistical MST toolbox approach. The library was constructed to include both CUPs and ARPs, determined using the Biolog™ Microbial Identification System, and the Kirby-Bauer disk diffusion method with the Biomic™ automated reading system. In addition to CUPs, the Biolog system can generate enterococcal identification down to the species level providing additional information about possible host sources, as some *Enterococcus* spp. can be associated with particular hosts and environments (Devriese *et al*. 1987; Wheeler *et al*. 2002; Scott *et al*. 2005; Moore *et al*. 2006). ARPs coupled with CSU profiles, can be used to create fingerprints that can generate a characteristic profile for strains from various host and environmental sources (Choi *et al*. 2003).

The known-source library was tested for accuracy and representativeness and was then compared to CUPs and ARPs of unknown source bacterial isolates from the upper section of Oso Creek using both discriminant analysis and

Random Forests to analyze the data and identify the main sources of fecal contamination in this portion of the creek. The data will be provided to policy makers for use in developing remediation strategies and best management practices to restore the water quality of Oso Creek.

OBJECTIVES

The purpose of this study was to determine the animal sources of enterococci in the upper portion of Oso Creek using two phenotypic MST methods: carbon source utilization and antibiotic resistance profiling. This goal was accomplished via the following objectives:

1. Conduct representative field sampling of both animal and human fecal sources that potentially contribute to the contamination of Oso Creek, and isolate *Enterococcus* spp. using selective microbiological methods.
2. Construct a known-source library of *Enterococcus* profiles comprising species level identification, carbon source utilization profiles and antibiotic resistance profiles.
3. Isolate enterococci from water and sediments of the upper Oso Creek and establish profiles comprising species level identification, carbon source utilization profiles, and antibiotic resistance profiles.
4. Use two statistical techniques, linear discriminant analysis and Random Forests, to compare profiles of the known-source library isolates with those of creek enterococci to determine sources of fecal contamination in Oso Creek.

STUDY SITE

The study site is defined as the upper Oso Creek watershed (Figure 1). Animal fecal samples were collected from within this watershed, while enterococci were isolated from water and sediment samples collected directly from the upper portions of Oso Creek and West Oso Creek. Oso Creek is a small 12.8 km^2, effluent-dominated, low-gradient stream located in the Nueces-Rio Grande Coastal Basin (TCEQ Basin 22) and is defined by the Texas Water Quality Inventory and 303(d) List as Segment 2485A (Withers and Chapman 1993; TCEQ 2002, 2010). The Oso Creek watershed comprises approximately 234 km^2 of the 609 km^2 basin (Hay and Mott 2007; TCEQ 2007).

Oso Creek begins 4.8 km upstream of State Highway 44 near the Robstown Waste Water Treatment Facility (RWWTF), west of Corpus Christi in Nueces County, and runs 47.5 km southeast until reaching confluence with Oso Bay in southern Corpus Christi (TCEQ 2005; Hay and Mott 2007). There is approximately 23 km of non-tidal freshwater flowing into a 17 km tidal portion of the creek. Oso Creek is the primary freshwater source for the 18 km^2 Oso Bay, and is the main drainage channel for more than 96 km of natural and constructed drainage (Nicolau 2001; Hay and Mott 2005; TCEQ 2010). Approximately 18.3 km downstream of Oso Creek, a stream formed from runoff, called West Oso Creek, joins with the main Oso Creek. West Oso Creek flows across Farm to Market 665 and through cow and horse pasture (Mott and Hay 2008). Since April 1972, Oso Bay has been assayed for fecal indicator bacteria.

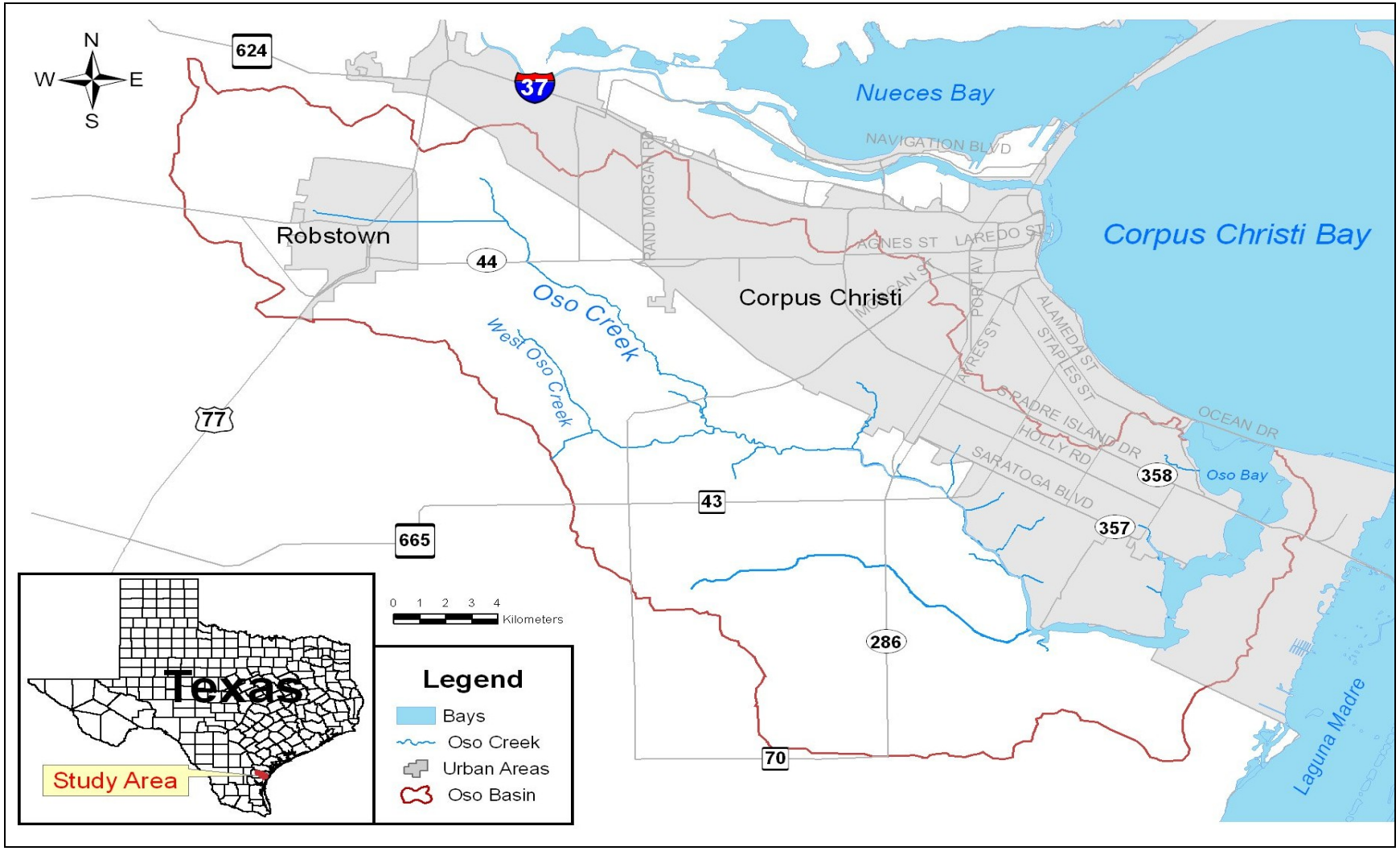

Figure 1 Study site: Oso watershed

Since 2006, Oso Bay itself has been listed as impaired due to elevated levels of fecal bacteria in its shellfish harvesting waters (Crysup 2002; TCEQ 2010). The shallow Oso Bay (depth <1.0 m) is classified as "exceptional aquatic habitat" (Nicolau 2001), supporting many plants such as the seagrass *Halodule beaudettei,* along with a range of vertebrates and invertebrates including the southern flounder (*Paralichthys lethostigma*), white and brown shrimp (*Litopenaeus setiferus* and *Farfantepenaeus aztecus*), and blue crabs (*Callinectes sapidus*). Oso Bay also provides recreational fishing, tourism benefits, and plays a significant role in water purification and storm protection for both the city of Corpus Christi and Nueces County, Texas (Hildebrand and King 1979; TNRCC 1996; Nicolau 2001). Oso Bay is a secondary bay, exchanging estuarine water along its southern shore with the Corpus Christi Bay system, which, according to the US EPA, has been designated as an estuary of national significance (US EPA 1999; Nicolau 2001).

Topographically, the Oso watershed is flat to gently sloping with remnants of Pleistocene marine terraces. From the inception of Oso Creek northwest of Robstown, the total change in elevation of the creek to the confluence with Oso Bay within the basin is approximately 28 m, for an overall change in slope of about 0.7m/km (Hay and Mott 2005). The Oso watershed lies on the Pleistocene Beaumont Formation, which is largely composed of low permeability interdistributary muds, fluvial over-bank muds, and idle channel-filled muds. The rest of the basin is composed of low-to-moderate permeability of crevasse splay, meander belt, levee, and distributary sand deposits (Hay and Mott 2005).

Sediments within Oso Creek are comprised of soft organic mud, silts, and clays. The soils surrounding Oso Creek are composed of three types: Victoria Association, Orelia-Banquete Association, and Galveston-Mustang-Tidal Flats Association (USDA 1992). West Oso Creek is dominated by cultivated, pastured, and rural lands. The majority of land in the Oso watershed is planted or cultivated (67.8 %), with a lesser amount being urban and suburban developments (13.8 %) (Table 1) (Hay and Mott 2005).

Table 1 Land use in the Oso Bay/Oso Creek watershed

Land Use Types	Percent (%)
Planted/Cultivated	67.8
Urban Development	13.8
Grasslands	5.2
Water	4.5
Shrubland	3.8
Wetlands	2.8
Forested Upland	2.0
Barren	0.2

Adapted from Hay and Mott 2005.

Water depth varies within the creek from 0.20 m to 0.75 m in flowing runs and pools up to 1.5 m deep. Very little flow is attributed to the creek; with flow often 0.08 m^3 s^{-1} in running areas (NOAA 2011). The creek, however, has reached flood stage (>6.1 m) 22 times since 1980, with no recorded floods in 2009, and three major flooding events recorded throughout 2010 (cresting on: 1/16/10, 7/3/10, and 9/20/10) (NOAA 2011). Flooding results in lowland flooding of farm and ranch lands, suburban areas, golf courses, primary highways,

secondary roads, and low bridges (NOAA 2011). The mean annual precipitation for the watershed averages 74 cm y^{-1}, with yearly precipitation in 2009 and 2010 totaling 52.3 cm and 111.6 cm respectively (Nicolau 2001; NOAA 2009, 2010). Oso Creek is an area of semi-arid and sub-tropical climate, with higher than average annual moisture deficits, hot, humid summers and mild, cool winters, and mean annual evaporation rates between 90 to 115 cm yr^{-1} (Nicolau 2001).

The Oso watershed has several identified outflows permitted by TCEQ with allotted specified discharge limits. Along Oso Creek, entities including the City of Robstown, Equistar Chemical LP Corpus Christi Plant, Texas A&M University Agriculture Research Extension, City of Corpus Christi Greenwood Waste Water Treatment Plant, and the City of Corpus Christi Storm Water all have permitted outflows, regulated by TCEQ (Table 2; Figure 2). However, high concentrations of fecal bacteria have been detected in the upstream portions of Oso Creek during dry weather sampling events and sources of these fecal bacteria remain unknown (Hay and Mott, 2005).

Table 2 Permitted discharges in the Oso watershed

Permitted Discharger	TCEQ Permit No.	Permitted Daily Avg. Flow (MGD)
American Electric and Power Barney Davis Power Station	01490-000	540.00
City of Corpus Christi Oso WWTP	10401-004	16.200
City of Corpus Christi Greenwood WWTP	10401-003	8.0000
Texas A&M University CBI La Coss Facility	03646-000	5.0400
City of Robstown	10261-001	2.4000
Equistar Chemical LP Corpus Christi Plant	02075-003	2.0000
Tennessee Pipeline Construction Co. Cuddihy Airfield WWTP	14228-001	0.0600
Corpus Christi Peoples Baptist Church Roloff WWTP	11134-001	0.0200
Texas A&M University Agriculture Research Ext.	11345-001	0.0015
City of Corpus Christi Storm Water	04200-000	NA

Adapted from Hay and Mott, 2005.

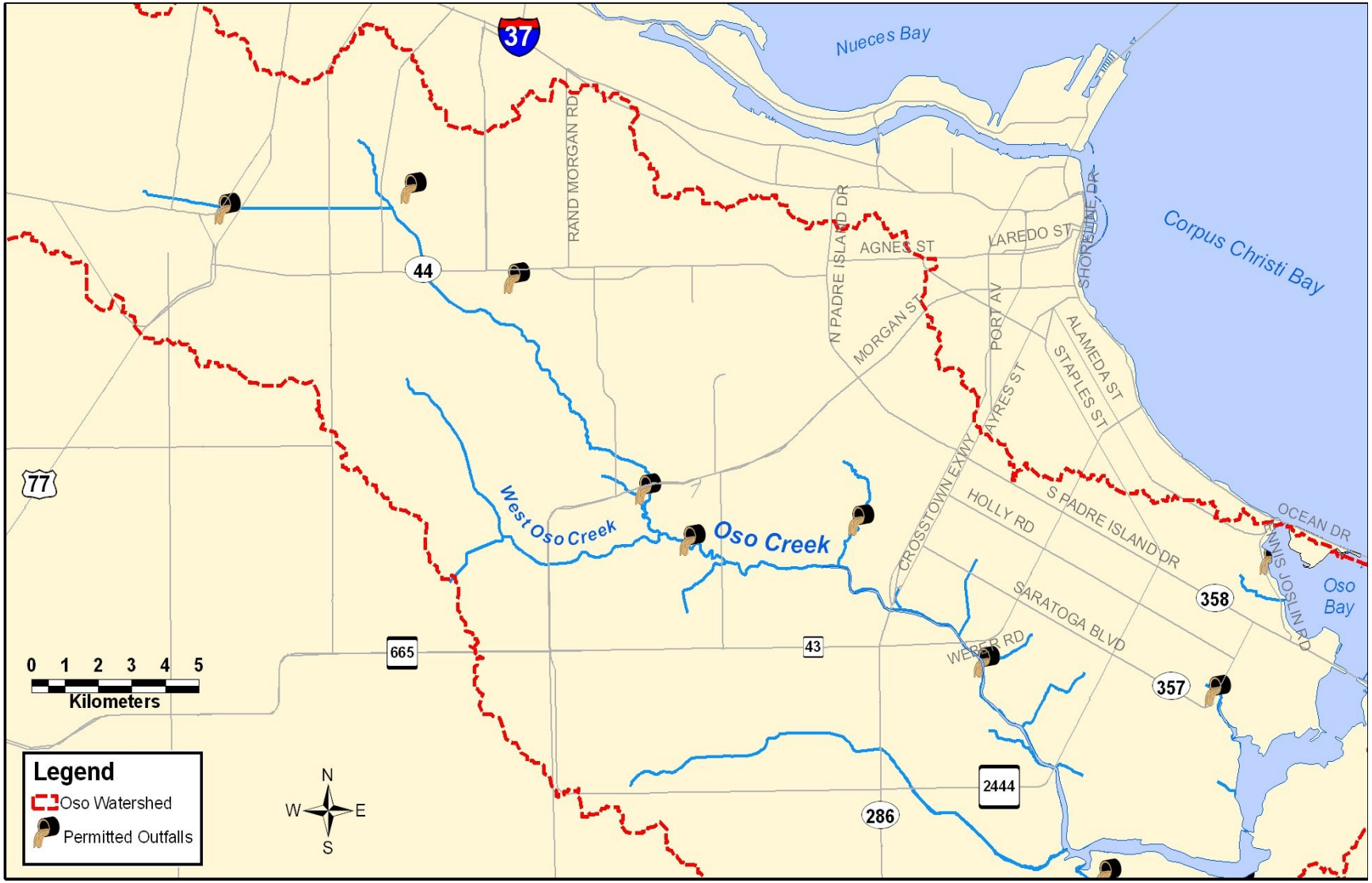

Figure 2 Permitted discharges in the Oso watershed

MATERIALS AND METHODS

Methods followed those described in the Quality Assurance Project Plan (QAPP) of the Project "Identify and Characterize Nonpoint Source Bacteria Pollution Support Implementation of Bacteria TMDLs in the Oso Bay Watershed" Texas State and Soil Water Conservation Board Project 07-13 (Hay and Mott 2008).

Field Collection of Fecal Samples

Determination of Fecal Sources

Land use and sanitary surveys conducted by Hay and Mott in 2005, together with Oso watershed field surveys conducted during this study, were used to identify possible sampling sites within the Oso watershed for various animals. This information, in conjunction with input from Oso Creek/Oso Bay TMDL Stakeholders, United States Department of Agriculture, and Texas Parks and Wildlife Department (TPWD), was used to develop a list of potential animal sources and sample locations. Based on current literature, human, livestock, wildlife (both avian and non-avian), and to a lesser extent domestic animals, may play significant roles in contamination of rural creeks dominated by agricultural and livestock farmland (Edwards *et al*. 2000; Whitlock *et al*. 2002; Choi *et al*. 2003; Kelsey *et al*. 2003; Graves *et al*. 2007; Somarelli *et al*. 2007; Vogel *et al*. 2007; Smith 2009).

For this study a total of 21 human, 15 domestic animal, 43 avian wildlife, 46 non-avian wildlife, and 77 livestock fecal samples were collected for isolation

of *Enterococcus*. Fecal sampling focused on the upper portion of Oso Creek from the RWWTF to Naval Outlying Field Cabaniss, Corpus Christi, Texas; however, several sites within the Oso watershed, along the creek, and attached secondary drainage creeks were sampled (Figure 3; see Appendix A, Table 1 for additional information). Sampling of animal sources began on 10/27/2009 and continued until 3/4/2011 with fourteen sampling events in total, spread out over the course of the ~1.5 year sampling period. To note, one sampling site was located outside the Oso watershed; however, it was a veterinary establishment that boards animals from within the sampling site (Figure 3) (Appendix A, Table 1).

Human/Sewage Sampling

Aseptic technique was employed at all times, with double gloves and protective eye and body wear worn during collection of raw sewage samples. Human fecal samples were collected as both raw (influent) and treated (effluent) sewage from the RWWTF influent intake tower and effluent discharge pipe (Figure 3, 9). Septic systems within the watershed with pump-out fecal material were not available to be sampled for this project. Autoclaved, sterile, polypropylene screw-capped Nalgene® (16067-124; Thermo Scientific Inc., Rochester, NY) bottles were used for both influent and effluent wastewater. Effluent wastewater samples were collected aseptically at the mouth of an effluent discharge pipe within a drainage canal, adjacent to the RWWTF, which flows into Oso Creek (TCEQ 2005).

Figure 3 Sampling locations of animal fecal material

Influent samples were taken by inserting a Sludge Nabber and Swing Sampler extension arm (57581; Lab Safety Supply, Janesville, WI) with a sterile, Nalgene® bottle attached to it, into the facilities influent intake tower. Influent sample bottles were shaken to assure an even suspension of microorganisms in the wastewater column according to standard methods (TCEQ 2003; APHA 2005). BD BBL™ CultureSwab™ EZ (220144; Beckton, Dickinson and Company, Sparks, MD) sterile, polyurethane tipped swabs were then inoculated on-site by dipping the swab tip into the influent wastewater suspension. Swabs were replaced into their respective sterile containers; both containers and field data sheets were labeled with appropriate information (Appendix B, section a). For safety purposes, influent sample containers and tips were double bagged in biosafety bags, and placed in shock- resistant biosafety liquid sealed *Infecon 3000 Infectious Substance Shippers – 6.2 U.N. Certified* canisters (INF-3000; Medical Products, Com-Pac International, Inc., Carbondale, IL) before being stored on ice at approximately 4 °C. These samples were accompanied by hazardous material safety handling instructions (as per project QAPP) during transportation from field to lab for analysis (Appendix B, section b).

Domestic, Livestock, and Non-Avian Wildlife Sampling

Aseptic technique was employed at all times with gloves being worn during collection. Fecal matter from livestock was collected at ranches, stables, and farms (Figure 3). Fecal matter from domestic animals was collected at various kennels, veterinary establishments, and animal shelters. Fecal matter from non-avian wildlife was collected using medium wire traps baited with cans of

tuna as well as via 'road kill' sampling. Assistance from local hunters as well as TPWD mammalogist, Dr. John Young, was provided during trapping sessions. Samples from deceased non-avian wildlife (*i.e.* road-kill) were collected by puncturing the animal with a sterile scalpel and swabbing the intestinal portion by rolling the sample tip of a sterile BD BBL™ CultureSwab™ EZ within the carcass. Domestic, livestock, and non-avian wildlife animal droppings were sampled by rolling the sample tip of a sterile BD BBL™ CultureSwab™ EZ within a fresh fecal sample. Autoclaved, sterile, tongue depressors were used to scrape off the exterior, potentially contaminated portion of the fecal samples, before collection of a sample, in order to obtain a pure sample from within the deposit. The swab was replaced into the sterile container, and these receptacles along with field data sheets were labeled with appropriate information (Appendix B, section a). Containers were stored on ice at approximately 4 °C for transport back to the laboratory for analysis.

Avian Wildlife Sampling

Aseptic technique was employed at all times with gloves being worn during collection. Fecal matter from avian wildlife was collected with assistance from local hunters, TPWD mammalogist, Dr. John Young, and local TPWD game wardens. A tarpaulin method, described in previous projects (Stewart 2005) was initially used for the collection of avian droppings. This involved setting out a tarp, disinfecting it with Sporicidin® (Sporicidin International, Rockville, MD), and placing birdseed, chips, bread, or aromatic foods such as tuna or sardines on the surface of the tarp for the birds to feed on. Once the bird deposited fecal matter,

the tip of a sterile BD BBL™ CultureSwab™ EZ was rolled over the surface of the sample. Care was taken when swabbing the fecal sample, to avoid swabbing the white portion of the bird dropping, which contains uric acid.

Initial sampling events, however, yielded few samples utilizing the tarpaulin method. Mist netting was then employed with the help of TPWD. A fine mesh net was set between two 3 m high poles in front of potential bird nesting locations. Once the net was deployed, birds flew into the net and got caught within the nets' pouches for extraction. Birds were removed without harm and held upside down with hind feathers pushed askew to reveal the cloaca. The cloaca was then swabbed and the bird was released. On other occasions hand nets were used along Oso Creek water banks with similar collection techniques employed for sampling the cloaca. Larger birds, proved difficult to collect using tarpaulins, mist nets, or hand nets, and were shot by local hunters, TPWD mammalogist Dr. John Young, and field samplers. All hunters and samplers had current TPWD hunting licenses, with TPWD mammalogist Dr. John Young, possessing at the times of sampling, a special TPWD collections permit for all non-endangered avian species. Bird carcasses were sampled as described above, and then buried. Sample swabs were replaced into the sterile containers, and these receptacles along with field data sheets were labeled with appropriate information (Appendix B, section a). Containers were stored on ice at approximately 4 °C for transport back to lab for analysis.

Isolation of Enterococci from Fecal Samples

Enterococci were isolated from fecal samples via inoculation of BD Difco™ m*Enterococcus* (mE) Agar (233320; Becton, Dickinson and Company, Sparks, MD) plates or mE Agar modified with Indoxyl-β-D-Glucoside (mEI Agar) (I3450-1G; Sigma Aldrich, St. Louis, MO) with the fecally inoculated swabs. Several volumes of influent and effluent water were filtered (ranging from 0.1 mL to 100 mL) onto 0.45 µm cellulose nitrate filters (Sartorius, Edgewood, NY), with membrane filters then placed on mEI agar. For each wastewater event, any surplus influent or effluent wastewater was stored at 4 °C for up to 48 h in case of complications during the incubation of the first set of samples. Each plate was incubated at least either 24 h (mEI Agar) or 48 h (mE Agar) at 41 °C. Only single, isolated colonies that exhibited purple, maroon, or reddish brown hues on mE and black hues (without nitrocellulose membrane) or blue-haloed colonies (with nitrocellulose membrane) on mEI, were transferred, following standard microbiological isolation techniques as needed, to obtain a pure culture. If different morphologically (shape, elevation, size, color, form, margin, *etc*.) isolated colonies grew from initial fecal streaks, a representative sampling of these isolated colonies was transferred on through additional plating. Pure cultures were stored on BD Difco™ Tryptic Soy Agar (TSA) (236950; Becton, Dickinson and Company, Sparks, MD) slants after incubation for 18-24 h at 35.0°C. An initial goal of at least four isolates per sample was sought; however, up to twenty-five isolates were saved from some samples and in some cases, no isolates were cultivated on initial fecal streak plates.

Field Collection and Processing of Water and Sediment Samples

According to the US EPA, water bodies are generally not well mixed and thus a single sample is not representative of the entire water body. With regard to lotic situations, short-term variability at sampling locations can occur due to physical factors, weather, or season (*e.g.*, presence of transient or migratory animal populations), which can influence or skew bacterial levels of specific sites or samples (US EPA 2005; Smith *et al*. 2010). The US EPA therefore recommends taking several replicate samples or compositing samples over time (US EPA 2005). Since Oso Creek exhibits a large range of flow rates (Nicolau 2001), several water and sediment samples were taken quarterly over the course of a two-year period across at a number of locations in the creek in order to minimize variability and increase sampling representativeness.

Water samples were collected directly from the upstream portions of Oso Creek using autoclaved, sterile, polypropylene screw-capped Nalgene® bottles following standard water sampling methods (TCEQ 2003; APHA 2005). Water samples were collected from five historic TCEQ stations 18499, 18500, 18501, 20198, and 20559; however, two of the stations were frequently dry and isolated enterococci could only be grown from three of the five historic stations (18499, 18500, 18501) (Table 3; Figures 4, 5, 6, 7). Sediment samples were collected using PVC corers (sterilized for 1 h using ultra-violet light), which were inserted directly into the Oso Creek bed from five TCEQ stations 18499, 18500, 18501, 20198, and 20559 (Table 3; Figures 4, 5, 6, 7, 8, 9). Water and sediment samples were gathered over several sampling events in order to obtain

approximately 800 unknown source enterococcal isolates. A total goal for water isolates was 200 isolates collected per station. This was accomplished by collecting 50 isolates per station from four seasonal sampling events (three dry and one wet event). The remaining 200 isolates were isolated from sediments with an initial goal of 50 isolates being obtained per station. Water samples were filtered, following the standard US EPA Method 1600 filtration method (US EPA 2002b), onto a 0.45 µm cellulose nitrate membrane filter which was placed onto mEI Agar for isolation of enterococci. Sediment samples were pre-treated by preparing a dilution series followed by wrist action shaking for 1 h with phosphate buffered dilution water, before filtering the supernatant as described for the water samples. Colonies obtained from both water and sediment mEI Agar plates were then processed similarly to isolation for fecal sample isolates. Surplus creek water or sediment was stored at 4 °C for 48 h before disposal. Once pure isolates were obtained they were cultured onto TSA slants for analysis and storage.

Table 3 Locations of TCEQ historic sampling stations

Station ID (OST-TCEQ ID)	Description	Latitude	Longitude
OST-18499	Oso Creek at SH 44	27.783250	-97.592430
OST-18500	Oso Creek at FM665	27.729470	-97.523570
OST-18501	West Oso Creek at FM 665	27.709360	-97.554220
OST-20198	Upstream West Oso Creek at Merritt Road	27.730559	-97.576944
OST-20559	Robstown Waste Water Treatment Facility drainage ditch US 77	27.800060	-97.646530

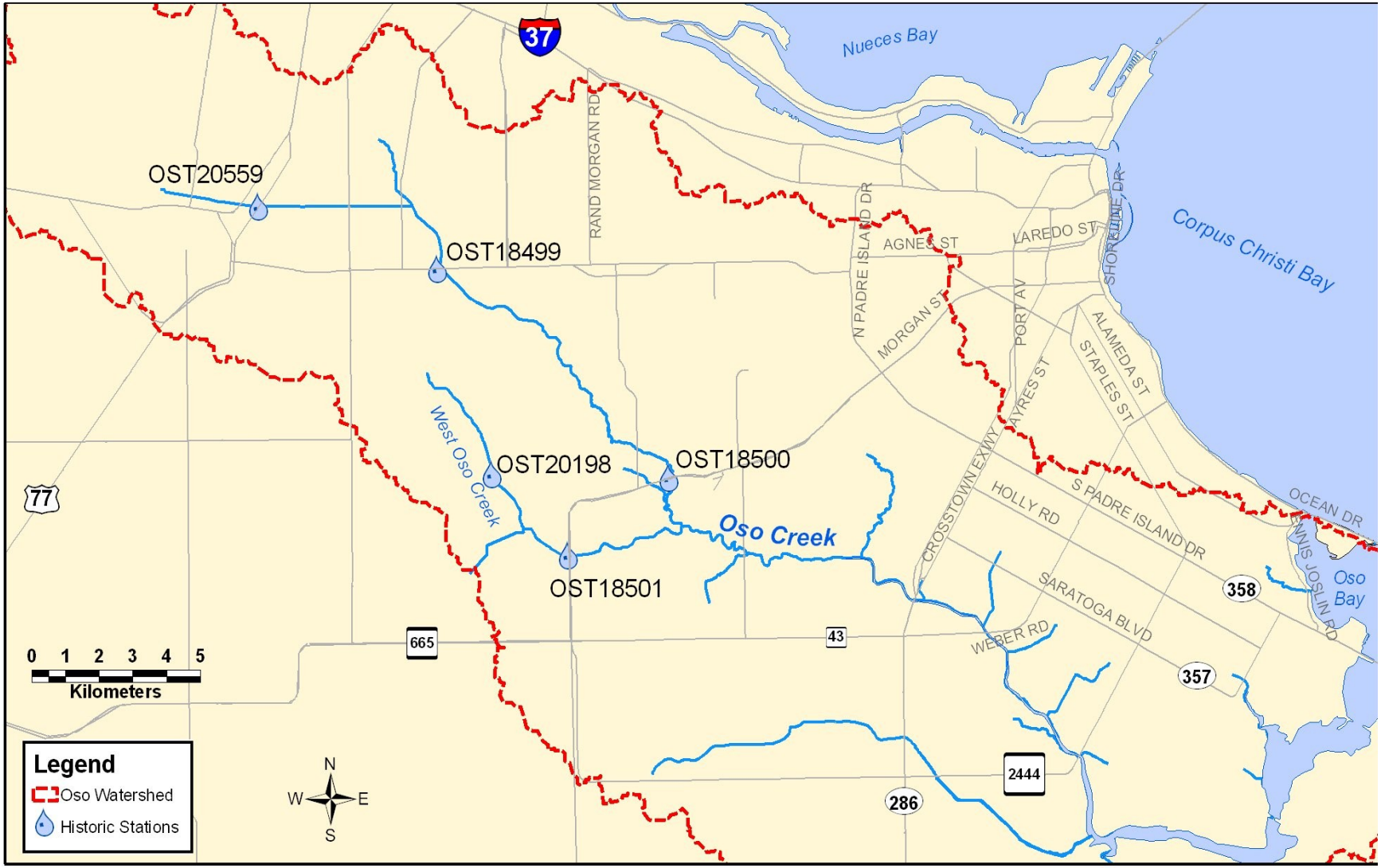

Figure 4 TCEQ historic stations in Oso watershed

Figure 5 TCEQ historic station 18499

Figure 6 TCEQ historic station 18500

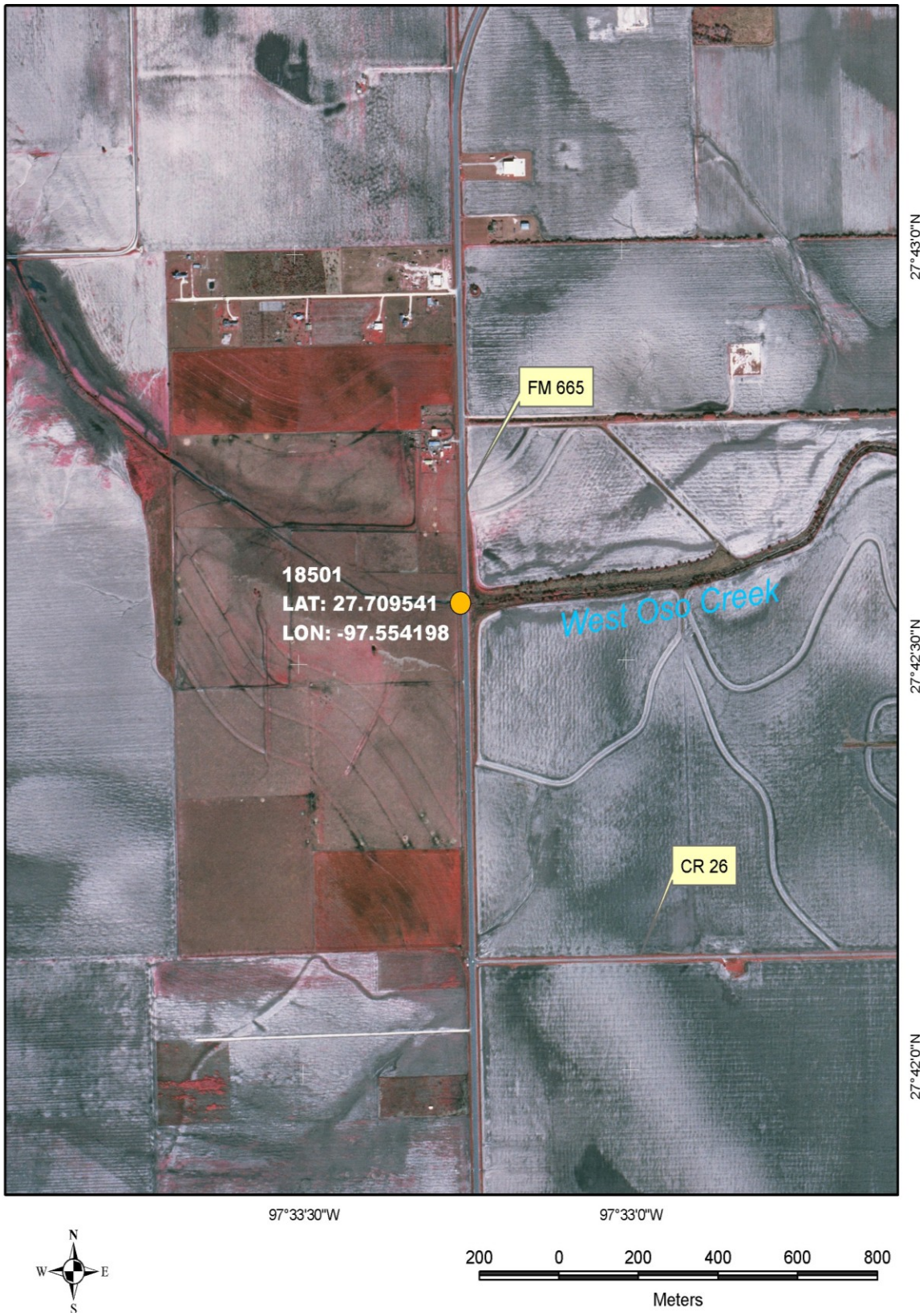

Figure 7 TCEQ historic station 18501

Figure 8 TCEQ historic station 20198

Figure 9 TCEQ historic station 20559

Analysis of Enterococci

Identification of fecal and creek enterococci species and characterization of their ARPs and CUPs followed EML standard operating procedures for CSU and ARP analyses, utilizing both the MicroLog™ (Biolog Inc.) Microbial Identification System and Kirby-Bauer disk diffusion with a Biomic™ automated image analysis system, as detailed in Appendix C. A previous *Enterococcus* library, consisting of 421 isolates, was developed in 2005 at Texas A&M University-Corpus Christi (Stewart 2005). This library contained *Enterococcus* CSU profiles of fecal isolates from human, cow, seagull, and dog; however, these isolates had not been analyzed for antibiotic resistance. The isolates from this library were re-grown from cryogenic preservation and antibiotic resistance profiles were developed in this study to add to the *Enterococcus* CSU and ARP data of isolates from fecal samples collected during this study.

Carbon Source Utilization Profiling

Briefly, pure cultures on TSA, from isolated colonies, were transferred to Biolog Universal Growth Medium supplemented with 5% Sheep's Blood (BUG/B) (71102; Biolog, Inc., Hayward, CA) and incubated at 35 ^{o}C for 24 h. Suspensions of each isolate were swabbed into inoculating fluid (0.4% NaCl, 0.03% Pluronic F-68, 0.01% Phytagel™) based on a turbidity of 20% T ± 2% at 600 nm. The resulting suspension was pipetted into a 96-well Biolog GP2 MicroPlate™, and plates were incubated for 24 h at 35 ^{o}C. After incubation, plates were read using the MicroLog™ Microbial Identification System, Release 4.20.04 (Biolog, Inc., Hayward, CA) (Biolog, 2004) to obtain color intensity and

+/- well reactions. Isolates that did not confirm to species level identification using the automated plate reader were also read manually to ensure correct identification. Isolates that identified only to the *Enterococcus* genus level were additionally tested on mEI agar for confirmation of *Enterococcus* genus. Isolates that exhibited a diffused black colony on mEI were recorded as belonging to the genus *Enterococcus* and further analyzed via ARP. Isolates that did not confirm as genus level *Enterococcus* were not further analyzed, nor were they included in cryopreservation. Isolates that did not identify as *Enterococcus* were most commonly identified as *Lactococcus* spp*., Pediococcus* spp*., Streptococcus* spp*., Alliococcus otitis, Staphylococcus* spp*., and Vagococcus lutrae*.

Antibiotic Resistance Profiling

Briefly, pure cultures on TSA from isolated colonies were streaked for isolation onto TSA plates. Up to four isolated colonies were selected from these streak plates to inoculate 5 ml tubes of prepared TSB. After 6 h of incubation, inoculated TSB suspension was added to blanked TSB-filled cuvettes until absorbency was between 0.08 and 0.10 at 625 nm. Once proper absorbency was achieved, each suspension was plated using a triple-lawn streak onto two Mueller Hinton Agar I (90006-573, Becton, Dickinson and Company, Sparks, MD) plates utilizing the standardized Kirby-Bauer disk diffusion method (Clinical and Laboratory Standards Institute 2006a, 2006b, 2008). A panel of twenty-one different antibiotics, as commercially prepared disks (BD BBL Sensi-Disc Antibiotics; Becton, Dickinson and Company, Sparks, MD) (Table 4) was dispensed using two sample plates (10 and 11 antibiotics respectively per plate)

and the plates were incubated for 18-24 h at 35 ^{o}C. Antibiotics were selected based on the suggested grouping of antimicrobial agents and interpretive criteria for disk diffusion and dilution susceptibility testing for *Enterococcus* species according to the Clinical and Laboratory Standards Institute (CLSI) and the recommendation of Facklam *et al.* (Facklam *et al.* 2002; CLSI 2006a, 2006b, 2008). Additionally, antibiotics were selected to include representatives from different groups of antibiotics and different uses among various animals. Diameters of zones of inhibition were measured in mm along with susceptibility (S), intermediate (I), and resistant (R) patterns (S-I-R patterns) (Table 5), using the Biomic™ Vision Microbiology Analyzer (Giles Scientific Inc., Santa Barbara, CA) to ensure uniformity for future comparisons with *Enterococcus* isolates from unknown sources.

Table 4 Antibiotics used to develop Antibiotic Resistance Profiles for *Enterococcus* isolates

Antibiotic	Abbreviation	Concentration
Ampicillin	AM	10 µg
Augmentin (Amoxicillin/Clavulanic Acid)	AmC	30 µg
Cefazolin	CZ	30 µg
Cefotaxime	CTX	30 µg
Ceftazidime	CAZ	30 µg
Ceftriaxone	CRO	30 µg
Chloramphenicol	C	30 µg
Ciprofloxacin	CIP	5 µg
Doxycycline	D	30 mg
Enrofloxacin	ENO	5 µg
Gentamicin	GM	10 µg
Imipenem	IPM	10 µg
Kanamycin	K	30 µg
Nalidixic acid	NA	30 µg
Neomycin	N	30 µg
Spectinomycin	SPT	100 µg
Streptomycin	S	10 µg
Sulfamethoxazole / Trimethoprim	SXT	23.75 µg / 1.25 µg
Sulfisoxazole	G	0.25 mg
Tetracycline	Te	30 µg
Vancomycin	V	30 µg

Table 5 Susceptible (S), intermediate (I), and resistant (R) ranges (mm) for *Enterococcus* using Biomic™ Microbiology Vision Analyzer (2007 software)

Antibiotic	S	I	R
Ampicillin	≥17	14-16	≤13
Augmentin (Amoxicillin/Clavulanic Acid)	≥18	14-17	≤13
Cefazolin	≥18	15-17	≤14
Cefotaxime	≥23	15-22	≤14
Ceftazidime	≥18	15-17	≤14
Ceftriaxone	≥21	14-20	≤13
Chloramphenicol	≥18	13-17	≤12
Ciprofloxacin	≥21	16-20	≤15
Doxycycline	≥14	11-13	≤10
Enrofloxacin	≥21	16-20	≤15
Gentamicin	≥15	13-14	≤12
Imipenem	≥16	14-15	≤13
Kanamycin	≥18	14-17	≤13
Nalidixic acid	≥19	14-18	≤13
Neomycin	≥17	13-16	≤12
Spectinomycin	≥18	15-17	≤14
Streptomycin	≥15	12-14	≤11
Sulfamethoxazole / Trimethoprim	≥16	11-15	≤10
Sulfisoxazole	≥7	NA	≤6
Tetracycline	≥15	12-14	≤11
Vancomycin	≥17	15-16	≤14

Storage of Isolates

All *Enterococcus* isolates that were confirmed and speciated via the MicroLog™ Microbial Identification System were transferred to cryogenic storage vials (2 ml, 66008-284; VWR, West Chester, PA). The *Enterococcus* isolates were first transferred from the TSA slants to 5 ml of BD Difco™ Tryptic Soy Broth (TSB) (211825; Becton, Dickenson and Company, Sparks, MD). The cultures were placed in an incubator orbital and incubated at 35 °C for 16-18 h. After incubation, 600 µl of the overnight bacterial culture was pipetted into a cryovial, followed by the addition of 400 µl of sterile glycerol. After the glycerol was added, the sample was gently mixed and placed in a vial box holder. The vial boxes were then placed into a -80 °C freezer. Triplicate vials were made for each known animal source *Enterococcus* isolate with duplicate vials being placed in separate -80 °C freezers at Texas A&M University – Corpus Christi, Texas and the triplicate vial being additionally sent to James Madison University, Virginia.

Quality Assurance / Quality Controls

Proper sample handling and custody procedures ensured the custody and integrity of samples beginning at the time of sampling and continuing through transport, sample receipt, preparation, and analysis. A Chain of Custody (COC) form was used to document handling of samples during transfer from the field to the laboratory (Appendix B, section c). All samples were contained in 4 °C ice chests and held no longer than 18 h before processing. All fecal swabs were bagged and refrigerated at 4 °C in case of future complications. Wastewater samples were also tested using potassium iodide strips to test for chlorine levels

within the water. No readings above 0.01 ppt Cl were observed from any sampling event.

Quality control samples were run (*e.g.*, positive controls, negative controls, and blanks) for each selective medium lot, as well as positive controls and sterility checks for all batches of media as specified in S*tandard Methods for the Examination of Water and Wastewater*, 20th ed. American Public Health Association (APHA) (2005) and the 2003 National Environmental Laboratory Accreditation Program guidelines. Positive and negative control cultures for enterococci were used as per APHA (2005) with positive controls being *Enterococcus faecalis* ATCC 19433 and 29212, and negative controls being *Enterobacter aerogenes* ATCC 13048 and *Escherichia coli* ATCC 11775, with *Enterococcus faecalis* ATCC 19433 and *Enterobacter aerogenes* ATCC 13048 being used as positive and negative quality controls on mE and mEI agars. Requirements for these samples, their acceptance criteria, and corrective action are all method-specific as per the project QAPP. Media log sheets indicating date, medium, volume, pH, and lot numbers were kept for all prepared media. All inoculated plates, tubes, broths *etc*. were autoclaved in biohazard bags with indicator tape for at least 30 min at 121 °C prior to disposal. Media that supported the growth of negative controls did not support the growth of positive control, failed sterility checks, or failed pH values was discarded and remade.

Quality controls for CSU were followed according to the protocol described in the MicroLog™ System Release 4.0 User Guide (Biolog 1999). Each lot of BUG/B and GP2 MicroPlates™ had been tested for internal quality control

standards before being released for sale. Internal quality controls for BUG/B conducted by Gibson Laboratories, LLC., tested gel strength, bioburden performance, pH (7.3 ± 0.1), and biological performance utilizing *Streptococcus pyogenes* ATCC 19615, *Streptococcus pneumoniae* ATCC 6305, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922 (Gibson Laboratories, LLC, Lexington, KY). As per project QAPP and Biolog recommendations, a set of four gram-positive control strains were streaked onto BUG/B plates, inoculated onto GP2 MicroPlates™, and analyzed via the Biolog MicroStation™ Reader for quality control purposes with each analysis. These strains included *Corynebacterium minutissimum* ATCC 23348, *Rhodococcus equi* ATCC 6939, *Staphylococcus aureus* spp. *aureus* ATCC 12600, and *Enterococcus faecalis* ATCC 19433. If multiple controls failed to speciate correctly, the project Quality Assurance Officer (QAO) (Ms. LaDonna Henson, MS) was contacted and the samples corresponding to the quality control batch were discarded.

The recommended quality control for ARP was *Staphylococcus aureus* ATCC 25923, which had been determined by CLSI standards for acceptable zone diameters to certain antibiotics (CLSI 2006, 2008a, 2008b). The MicroLog™ Microbial Identification System performs performance and calibration checks upon each startup. The BIOMIC™ Vision Microbiology Analyzer includes calibration plates, which may be used to assay performance of the analyzer. These calibration plates were used every six months to ensure proper performance.

Laboratory Duplicates

Laboratory duplicates were performed on 5% of all isolates analyzed for CSU, with quality control organisms being duplicated or triplicated with each CSU run. Duplicates were performed on the BUG/B plating step, as well as the inoculation step of GP2 MicroPlate™. Acceptable duplicates resulted in similar matching of genus or species level identification of original isolate. Laboratory duplicates were performed on 10% of all ARP isolates, with the quality control organism duplicated or triplicated with each ARP run. An acceptable range in zone diameters for duplicates was considered ± 3 mm. If this range was exceeded for more than one antibiotic, data was then examined by project QAO as per project QAPP. Professional judgment was used to determine whether or not the sample had to be omitted from the database or if any other course of action was warranted.

Statistical Methods

Utilizing a phenotypic toolbox approach generated *Enterococcus* CSU and ARP data for this study. This study's known-source *Enterococcus* library was initially supplemented with Stewart's 421 *Enterococcus* isolates collected from human, seagull, dog, and cow samples from the Nueces County area (Stewart 2005). However, Stewart's cow and human isolates exhibited differences in profiles from current project isolates and several of Stewart's cow and human fecal samples were collected outside of the current study site. Inclusion of these isolates in the library also reduced discrimination among sources and average rates of correct classification. Based on these factors the cow and human

isolates were excluded from the final library, while the seagull and dog isolates were retained.

According to the US EPA, one of the currently accepted methods for statistically analyzing phenotypic data in MST is discriminant analysis (US EPA 2005). In order to examine the best possible approach and to limit misclassification, two statistical methods were used to analyze CSU and ARP data. Linear discriminant analysis (LDA) and Random Forests (RF) were used to construct the best possible models using the known-source data. Both statistical methods were analyzed for performance based on average rates of correct classification. Models examined were a two-way model (human vs. nonhuman), three-way model (human vs. domestic animal vs. wild animal), four-way model (human vs. livestock vs. dog vs. wild animal), five-way model (human vs. livestock vs. dog vs. seagull vs. wildlife [avian/non]), and seven-way model (human vs. cow vs. horse vs. dog vs. seagull vs. "other avian" vs. non-avian wildlife) (Table 6). These models were selected to provide a range of discrimination between human and non-human sources, as well as provide maximum discrimination among all animal types, with similar models utilized by previous MST studies (Casarez *et al.* 2007; Moussa and Massengale 2008; Smith 2009; Smith *et al.* 2010). Multiple models were run using the statistical packages SPSS® software edition 17.0 (2008) (SPSS Inc., Chicago, IL) for LDA utilizing equal prior probabilities for each group. This allowed the groups of known source isolates to be considered equally regardless of sample size (*e.g.*

for the human vs. non-human model the two groups were very unequal in size) and to ensure the representativeness of the library.

The library was also challenged with a subset of 20% of the CUPs and ARPs being excluded from the library as a test set. The five models were then analyzed using the remaining 80% of the library ("the training set"), and the test set was then analyzed as an unknown source. A representative analysis should produce similar results with the test set as with the full library. Additionally, cross-validation analysis (jackknifing), a function of SPSS and other statistical programs, removed each isolate one at a time and classified it according to the remaining isolates. The US EPA guidelines recommend that for a library to be considered representative, LDA should produce no more than a 5% average rate of correct classification difference between originally run data models and cross-validated models (US EPA 2005).

Table 6 Classification models for statistical analysis

	Models
2-Way Classification	Human vs. Non-Human
3-Way Classification	Human vs. Domestic Animal vs. Wild Animal
4-Way Classification	Human vs. Livestock vs. Dog vs. Wild Animal
5-Way Classification	Human vs. Livestock vs. Dog vs. Seagull vs. Wildlife (Avian/Non)
7-Way Classification	Human vs. Cow vs. Horse vs. Dog vs. Seagull vs. "Other Avian" vs. Non-Avian Wildlife

R software 2.13.0 (2011) (R Foundation for Statistical Computing, Vienna, Austria) was utilized for RF, validation of LDA and RF, and for variables of importance classification (data not shown). The library "randomForest" was utilized, along with structured code by Dr. Blair Sterba-Boatwright (Appendix D) that was adapted by Rodriguez to run both Random Forest and stratified Random Forest. Using stratified Random Forest allowed for sample sizes to have defined representation, giving underrepresented groups a higher selection rate during construction of the forest. Random Forest was tested using the same 80%-20% training and test set method to ensure the model did not overfit.

Models were also tested for temporal and geographic stability. The excluded cow and human isolates from Stewart's study were used to challenge the constructed library due to the fact that Stewart's isolates were collected in 2005 (temporal stability) and that his cow and human isolates were collected from outside the Oso watershed (geographic stability).

Once models using the known source isolates had been constructed and tested for accuracy, the CUPs and ARPs of isolates from unknown sources (Oso Creek sediment and water samples) were added to the analysis. Using LDA and RF, the five models were used to classify unknown isolates into the categories in order to discriminate sources of contamination in Oso Creek.

RESULTS

A summary of fecal sample sources and collection information is shown in Table 7. Complete data on field collection of each fecal sample is included in Appendices C and F. Field data sheets are stored at Texas A&M University – Corpus Christi. Complete spreadsheets of CUPs and ARPs, together with individual laboratory bacteriological analysis sheets for the Biolog GP2 MicroPlate™ well printouts, S-I-R patterns, and zone diameters generated by the Biomic™ Vision Microbiology Analyzer, are stored electronically at Texas A&M University – Corpus Christi.

Speciation of *Enterococcus* from Animal and Creek Sources

In order to construct and develop a library of known animal source enterococci, a total of 948 *Enterococcus* identified isolates were obtained from 202 animal fecal samples. Of these 948 isolates, 810 were identified to the species level with 90% or more phenotypic certainty using the MicroLog™ Microbial Identification System (the remaining 63 were identified to the genus level) (Table 7). Additionally, 421 enterococci from a previous CSU library were incorporated to expand the Oso watershed CSU enterococcal library to 1,369 isolates (data not shown) (Stewart 2005). Stewart's library contained *Enterococcus* CUPs of fecal isolates from human, cow, seagull, and dog. For a specific animal listing see Appendix E.

The known-source library developed in this study consisted of a total of 11 different *Enterococcus* species with the largest proportion (46.8%) of the isolates identified as *E. faecalis*. Two additional species isolated from animal fecal

samples, that when combined accounted for another 25% of the library, were *E. casseliflavus* and *E. faecium* (13.6% and 12.5% respectively) (Table 8).

Table 7 Summary of numbers of animal samples and *Enterococcus* isolates collected in the study

Animal Source	Number of Fecal Samples	Number Identified* as *Enterococcus*	Number Identified* to Species	Number *Enterococcus* ARA Profiled
Bird	43	291	266	276
Dog	15	69	58	60
Cow	53	162	118	151
Horse	24	109	95	92
Human	21	118	87	111
Non-Avian Wildlife	46	199	186	183
Total	202	948	810	873

* as determined using the MicroLog™ Microbial Identification System

Enterococcus casseliflavus and *E. faecalis* were the most common species isolated from human sources (21.84% and 20.69%), while *E. faecalis* constituted the largest proportion of isolates from non-human sources (49.79%) (Table 9). *E. pseudoavium* and *E. malodoratus* were only found in small numbers, and exclusively in human sources, while *E. solitarius* was exclusive to only non-human sources (Table 9). *E. faecalis* was isolated most frequently in bird and non-avian wildlife animal sources, while the majority of isolates from dog were *E. faecium* (63.8%) (Table 10). The lowest diversity of species was found in dog (five) while the greatest diversity was from human/sewage (nine). E,

faecalis, *E. faecium, E. mundtii,* and *E. gallinarum* were identified from all six animal sources (Table 10; Figure 10).

Table 8 Species, number of isolates, and percentages of each *Enterococcus* species isolated from animal sources

Species	# Isolates	Percentage (%)	Animal Sources
E. faecalis	379	46.8	Bird, Cow, Dog, Horse, Human, Non-Avian Wildlife
E. casseliflavus	110	13.6	Bird, Cow, Horse, Human, Non-Avian Wildlife
E. faecium	101	12.5	Bird, Cow, Dog, Horse, Human, Non-Avian Wildlife
E. mundtii	73	9.0	Bird, Cow, Dog, Horse, Human, Non-Avian Wildlife
E. flavescens	69	8.5	Bird, Cow, Horse, Human, Non-Avian Wildlife
E. gallinarum	57	7.0	Bird, Cow, Dog, Horse, Human, Non-Avian Wildlife
E. hirae	11	1.4	Bird, Cow, Dog, Horse, Non-Avian Wildlife
E. durans	4	0.5	Bird, Human
E. pseudoavium	4	0.5	Human
E. malodoratus	1	0.1	Human
E. solitarius	1	0.1	Horse

Table 9 Species of *Enterococcus* isolated from human compared with nonhuman sources

***Enterococcus* Species**	**Human Source**	
	# Isolates	**Percentage (%)**
E. casseliflavus	19	21.8
E. faecalis	18	20.7
E. gallinarum	15	17.2
E. flavescens	12	13.8
E. faecium	9	10.3
E. mundtii	8	9.2
E. pseudoavium	4	4.6
E. durans	1	1.2
E. malodoratus	1	1.2

***Enterococcus* Species**	**Non-Human Source**	
	# Isolates	**Percentage (%)**
E. faecalis	359	49.8
E. casseliflavus	91	12.6
E. faecium	92	12.8
E. mundtii	65	9.0
E. flavescens	57	7.9
E. gallinarum	42	5.8
E. hirae	11	1.5
E. durans	3	0.4
E. solitarius	1	0.1

Table 10 *Enterococcus* species isolated from each animal source group

Source	Species	# Isolates	Percentage (%)
Bird	*E. faecalis*	168	63.2
	E. mundtii	30	11.3
	E. faecium	28	10.5
	E. casseliflavus	16	6.0
	E. gallinarum	10	3.8
	E. flavescens	6	2.3
	E. hirae	5	1.9
	E. durans	3	1.1
Dog	*E. faecium*	37	63.8
	E. faecalis	13	22.4
	E. gallinarum	4	6.9
	E. mundtii	3	5.2
	E. hirae	1	1.7
Cow	*E. casseliflavus*	57	48.3
	E. flavescens	25	21.2
	E. faecalis	15	12.7
	E. gallinarum	8	6.8
	E. mundtii	7	5.9
	E. faecium	5	4.2
	E. hirae	1	0.9
Horse	*E. flavescens*	18	19.0
	E. faecalis	17	17.9
	E. faecium	17	17.9
	E. mundtii	16	16.8
	E. casseliflavus	13	13.7
	E. gallinarum	9	9.5
	E. hirae	4	4.2
	E. solitarius	1	1.1
Human	*E. casseliflavus*	19	21.8
	E. faecalis	18	20.7
	E. gallinarum	15	17.2
	E. flavescens	12	13.8
	E. faecium	9	10.3
	E. mundtii	8	9.2
	E. pseudoavium	4	4.6
	E. durans	1	1.2
	E. malodoratus	1	1.2
Non-Avian Wildlife	*E. faecalis*	146	78.5
	E. gallinarum	11	5.9
	E. mundtii	9	4.8
	E. flavescens	8	4.3
	E. casseliflavus	5	2.7
	E. faecium	5	2.7
	E. hirae	2	1.1

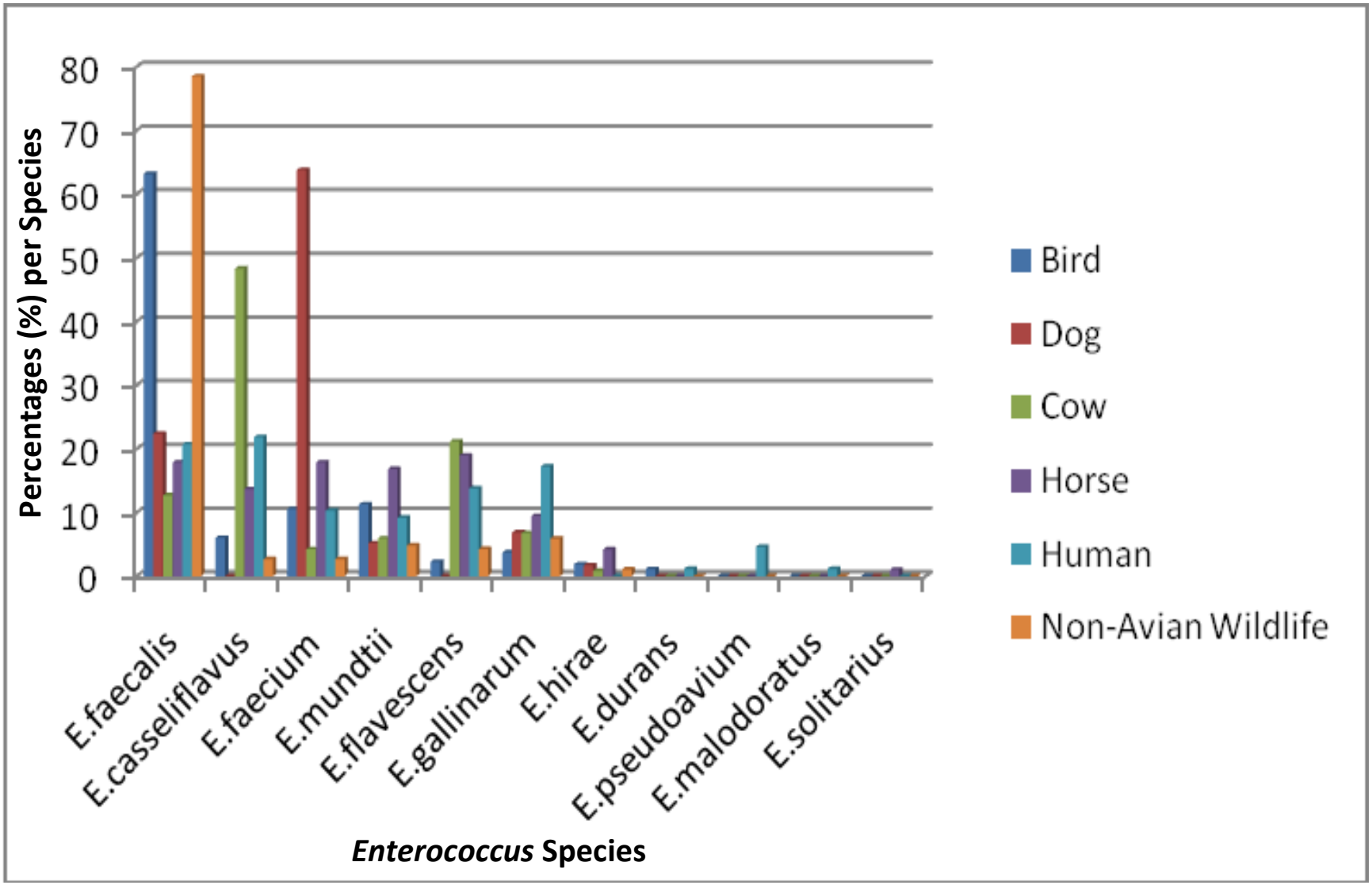

Figure 10 Percent of *Enterococcus* species isolated from animal sources

Unknown source enterococci were isolated from water and sediment samples collected from five different sampling locations (Table 3; Figure 4). A total of 824 *Enterococcus* isolates were obtained, with 740 isolates identifying to the species level via the MicroLog™ Microbial Identification System (Table 11). Sampling was conducted after rainfall (*i.e.* following runoff into the creek) and during dry weather, and isolates were analyzed from samples collected in each type of weather condition (Table 12).

Table 11 Summary of numbers of creek samples and *Enterococcus* isolates collected in the study

Source	Number Identified* as *Enterococcus*	Number Identified* to Species	Number of *Enterococcus* ARA Profiled
Sediments	211	161	193
Water	613	579	599
Total	824	740	792

* as determined using the MicroLog™ Microbial Identification System

Table 12 Summary of numbers of *Enterococcus* isolates collected from sediment and water samples during dry weather and following rainfall (wet events)

Source	Wet		
	Number Identified* as *Enterococcus*	***Enterococcus* Identified* to Species**	**Number of *Enterococcus* ARA Profiled**
Sediments	161	129	152
Water	222	204	215
Total	383	333	367
Source	**Dry**		
	Number Identified* as *Enterococcus*	***Enterococcus* Identified* to Species**	**Number of *Enterococcus* ARA Profiled**
Sediments	50	33	41
Water	391	374	384
Total	441	407	425

* as determined using the MicroLog™ Microbial Identification System

Enterococcus mundtii was the most common species isolated from Oso Creek water and sediments (45.4%), with *E. faecalis,* accounting for nearly a third of all the isolates (29.2%) (Table 13). Almost 75% of the creek isolates were identified as *E. mundtii* or *E. faecalis* whereas *E. faecalis* (46.8%), *E. casseliflavus (13.6%),* and *E. faecium* (12.5%) accounted for approximately 72% of animal enterococci isolates (Tables 8 and 13). Three species were isolated

from the creek in very low numbers, but not from any animal source: *E. dispar, E. raffinosus,* and *E. sulfureus,* the former two only one isolate each, in water, and the latter, four isolates, two each from water and sediments (Table 14).

The most prevalent enterococci isolated from Oso Creek water samples were *E. mundtii* and *E. faecalis* (48.6% and 32.3%) (Table 14) while in sediments, the most prevalent enterococcal species were *E. mundtii, E. faecium,* and *E. faecalis* (34.2%, 21.1%, and 18.0%) (Table 14).

Table 13 Species, number of isolates, and percentages of each *Enterococcus* species isolated from Oso Creek

Species	# Isolates	Percentage (%)
E. mundtii	336	45.4
E. faecalis	216	29.2
E. faecium	62	8.4
E. hirae	44	6.0
E. casseliflavus	26	3.5
E. gallinarum	27	3.7
E. flavescens	22	3.0
E. sulfureus	4	0.5
E. solitarius	1	0.1
E. raffinosus	1	0.1
E. dispar	1	0.1
Total	740	
Genus ID'ed	824	

During dry weather sampling events, over half of the enterococci isolated from both water and sediments were *E. mundtii* (57.4%) and 19.1% were *E. faecalis* (Table 14). In contrast, during wet sampling events, where rain exceeded 2 cm in the week preceding sampling (data not shown), 41.1% of the enterococci isolated from the creek were *E. faecalis*. *E. mundtii* was the second most frequently isolated species at 30.6% (Table 14). One isolate each of *E. raffinosus* and *E. dispar* were found in creek water during dry weather. *E. sulfureus* was also only isolated from the creek, (two isolates each from water and sediments), while one isolate of *E. solitarius* was collected from sediment during a dry event (Table 14).

Table 14 Species, number of isolates, and percentages of each *Enterococcus* species isolated from Oso Creek water and sediments during dry weather and following rainfall (wet events)

Source	Species	# Isolates	Percentage (%)
Creek Water	*E. mundtii*	281	48.5
	E. faecalis	187	32.3
	E. hirae	36	6.2
	E. faecium	28	4.8
	E. gallinarum	17	2.9
	E. casseliflavus	15	2.6
	E. flavescens	11	1.9
	E. sulfureus	2	0.4
	E. raffinosus	1	0.2
	E. dispar	1	0.2
Creek Sediments	*E. mundtii*	55	34.2
	E. faecium	34	21.1
	E. faecalis	29	18.0
	E. casseliflavus	11	6.8
	E. flavescens	11	6.8
	E. gallinarum	10	6.2
	E. hirae	8	5.0
	E. sulfureus	2	1.2
	E. solitarius	1	0.6
Dry Events	*E. mundtii*	234	57.4
	E. faecalis	78	19.1
	E. hirae	31	7.6
	E. faecium	27	6.6
	E. gallinarum	16	3.9
	E. casseliflavus	10	2.5
	E. flavescens	6	1.5
	E. sulfureus	3	0.7
	E. dispar	1	0.3
	E. raffinosus	1	0.3
Wet Events	*E. faecalis*	138	41.4
	E. mundtii	102	30.6
	E. faecium	35	10.5
	E. casseliflavus	16	4.8
	E. flavescens	16	4.8
	E. hirae	13	3.9
	E. gallinarum	11	3.3
	E. solitarius	1	0.3
	E. sulfureus	1	0.3

When the species of *Enterococcus* isolated from animals and from the creek were compared (Figure 11), *E. faecalis* was common in animal fecal samples and both creek water and sediment samples (>25% isolates from each source). Conversely, *E. mundtii* was isolated four times more frequently from creek samples than animal samples. Overall, animal sources contained higher percentages of *E. faecalis, E. faecium, E. flavescens, E. gallinarium,* and *E. casseliflavus*, but had lower percentages of *E. mundtii* and *E. hirae* (Figure 11).

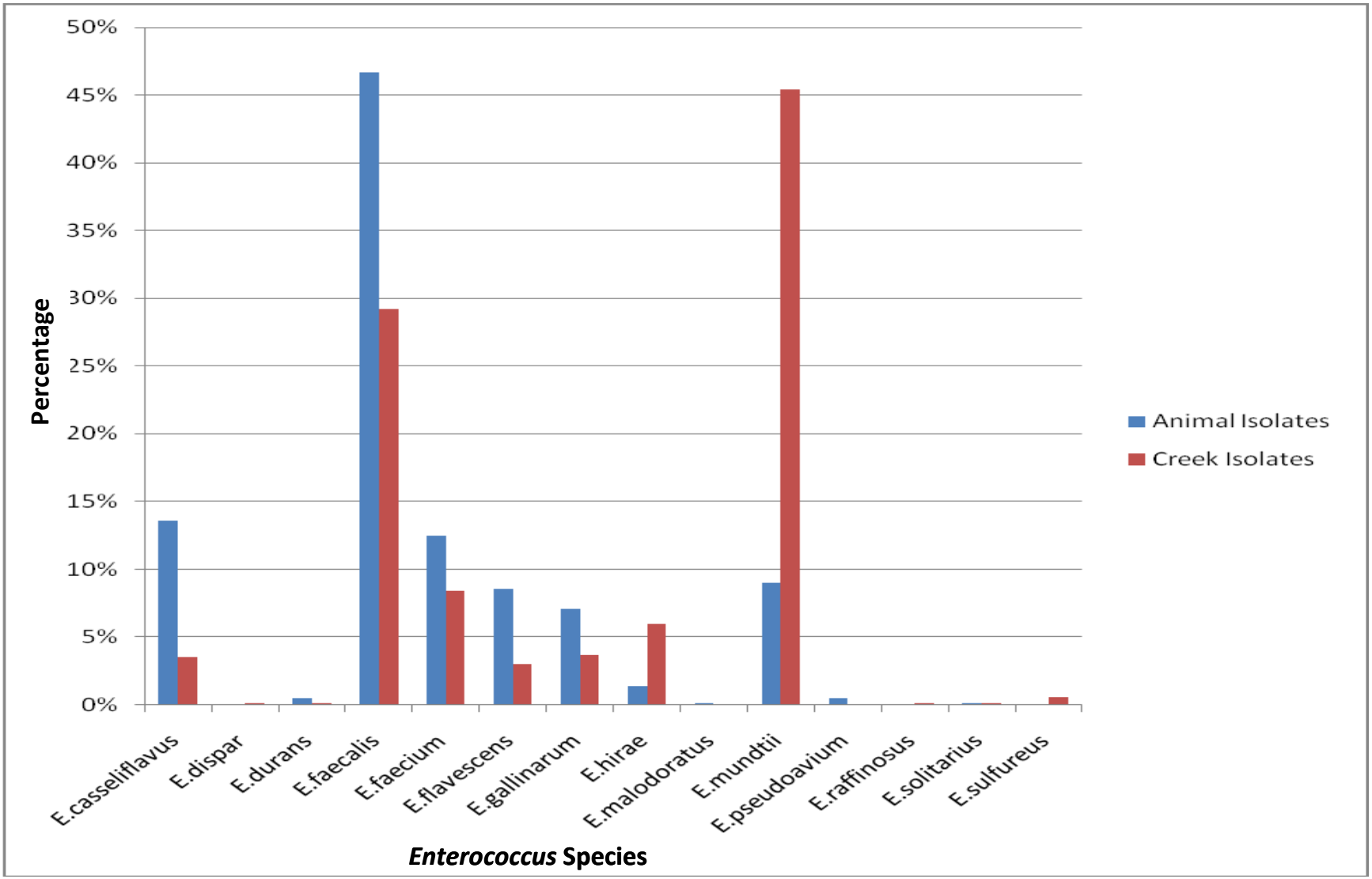

Figure 11 Comparison of *Enterococcus* species isolated from animal and creek sources

Construction of Known Source Library

The initial Oso Creek library consisted of carbon source utilization and antibiotic resistance profiles of 873 enterococci from animal sources obtained in this study (Table 7) and 378 enterococci isolated from Stewart's *Enterococcus* library (Stewart 2005). Combining and editing these two libraries formed a final library of 1085 CUPs and ARPs (Table 15).

The library included, as human source isolates, enterococci from effluent wastewater discharged directly into Oso Creek at the RWWTF and influent from the RWWTF influent wastewater tower. Stewart's human *Enterococcus* isolates from portable toilet and volunteer fecal material were excluded from this study, since these samples were collected in the Coastal Bend area but not all in the Oso watershed and additionally these sources of human material were unlikely to be main sources in the creek (compared with samples from the treatment plant whose outflow constitutes the main source of water comprising the upper creek). Cow and horse enterococci in the library were isolated from animals within the Oso watershed; Stewart's cow isolates were excluded since his fecal samples were collected from a local slaughter house and outside the watershed. Dog enterococci isolates were a combination of those collected in this study and Stewart's dog enterococci isolates to total 169 (Table 15). Seagull enterococci were derived solely from Stewart's previous study, while "other avian" and non-avian wildlife enterococci were those isolated from within the Oso watershed in this study. The final library used to detetermine sources of creek isolates therefore constituted 1085 isolates.

Table 15 Number of phenotypic profiles per animal source used in the known source library

Source	Number of Profiles (CUPs + ARPs) in Current Study	Number of Profiles (CUPs + ARPs) included from Stewart's Study	Total Number of Profiles (CUPs + ARPs)
Human	111	0	111
Cow	151	0	151
Horse	92	0	92
Dog	60	109	169
Seagull	0	103	103
Other Avian	276	0	276
Non-Avian Wildlife	183	0	183
Total	873	212	1085

Linear Discriminant Analysis Modeling of Library

The final library was analyzed using the five-model system described in the methods. For a two-way linear discriminant analysis model, (*i.e.* human vs. non-human) an average rate of correct classification (ARCC) of 92.5% was achieved with a leave-one-out cross-validation (jackknifing) ARCC of 89.6%. The rate of correct classification (RCC) for human was 85.6% and for non-human, 93.3% (Table 16). The cross-validation of 89.6%, suggests that the model was representative, since there was less than a 5% difference between original and cross-validated two-way models (US EPA 2005). The ARCC for the three-way model (human vs. domestic animal/livestock vs. wild animal) was lower, at 81.5% with an RCC of 83.8% for humans, 79.1% for domestic animal (cow, horse, and dog), and 82.7% for wild animal (bird and non-avian wildlife). The three-way model, however, had a 75.8% cross-validation, suggesting that the three-way model slightly overfit the data (difference of 6.9%) (Table 17). This loss of accuracy in discrimination was also reflected in the four-way classification.

The four-way classification model had a slightly lower ARCC of 81.2%. The RCCs were 79.3% for human, 78.2% for livestock, 88.8% for dog and 80.6% for wild animals. Humans had the largest drop in accuracy of classification from the three-way classification, with a decrease of 4.5%. The cross-validation for the four-way model was 73.3% with a difference of 7.9%, exceeding the recommended 5% rule and thus indicating a slight overfitting of the data (Table 18).

Table 16 Discriminant analysis of known source enterococci isolates from Oso Creek library. Two-way model—human vs. non-human (equal prior probabilities)

Classification Results

		Type	Predicted Group Membership		Total
			Human	Non-Human	
Original	Count	Human	95	16	111
		Non-Human	65	909	974
	%	Human	**85.6**	14.4	100.0
		Non-Human	6.7	**93.3**	100.0

a. 92.5% of original grouped cases correctly classified.

b. 89.6% of cross-validated grouped cases correctly classified.

Table 17 Discriminant analysis of known source enterococci isolates from Oso Creek library. Three-way model—human vs. domestic animal and livestock (cow, horse, dog) vs. wild animal (including bird) (equal prior probabilities)

Classification Results

		Type	Predicted Group Membership			Total
			Human	Domestic	Wild Animal	
Original	Count	Human	93	6	12	111
		Domestic Animal	26	326	60	412
		Wild Animal	17	80	465	562
	%	Human	**83.8**	5.4	10.8	100.0
		Domestic Animal	6.3	**79.1**	14.6	100.0
		Wild Animal	3.0	14.2	**82.7**	100.0

a. 81.5% of original grouped cases correctly classified.

b. 75.8% of cross-validated grouped cases correctly classified.

Table 18 Discriminant analysis of known source enterococci isolates from Oso Creek library. Four-way model—human vs. livestock vs. dog vs. wild animal (equal prior probabilities)

Classification Results

			Predicted Group Membership				
		Type	Human	Livestock	Dog	Wild Animal	Total
Original	Count	Human	88	6	4	13	111
		Livestock	18	190	9	26	243
		Dog	0	5	150	14	169
		Wild Animal	17	66	26	453	562
	%	Human	**79.3**	5.4	3.6	11.7	100.0
		Livestock	7.4	**78.2**	3.7	10.7	100.0
		Dog	.0	3.0	**88.8**	8.3	100.0
		Wild Animal	3.0	11.7	4.6	**80.6**	100.0

a. 81.2% of original grouped cases correctly classified.

b. 73.3% of cross-validated grouped cases correctly classified.

The five-way classification model increased slightly in terms of ARCC to 81.8% as compared to the four-way classification model. The RCC for human remained the same at 79.3%, with RCCs for livestock at 79.0%, dog at 82.2%, seagull at 87.4%, and wildlife (both avian and non-avian) at 82.6% (Table 19). Like the three- and four-way models, the five-way model had a cross-validation ARCC (73.5%) that differed more than 5% from the original model ARCC (81.8%) (Table19). The final model broke up animal groups into seven categories.

Table 19 Discriminant analysis of known source enterococci isolates from Oso Creek library. Five-way model—human vs. livestock vs. dog vs. seagull vs. wildlife (avian/non) (equal prior probabilities)

Classification Results

			Predicted Group Membership					
		Type	Human	Livestock	Dog	Seagull	Wildlife (Avian/Non)	Total
Original	Count	Human	88	8	3	0	12	111
		Livestock	16	192	9	0	26	243
		Dog	3	6	139	11	10	169
		Seagull	0	2	10	90	1	103
		Wildlife (Avian/Non)	16	57	7	0	379	459
	%	Human	**79.3**	7.2	2.7	.0	10.8	100.0
		Livestock	6.6	**79.0**	3.7	.0	10.7	100.0
		Dog	1.8	3.6	**82.2**	6.5	5.9	100.0
		Seagull	.0	1.9	9.7	**87.4**	1.0	100.0
		Wildlife (Avian/Non)	3.5	12.4	1.5	.0	**82.6**	100.0

a. 81.8% of original grouped cases correctly classified.

b. 73.5% of cross-validated grouped cases correctly classified.

The seven-way model had an overall ARCC of 77.4%; however, the cross-validation of 66.5% indicated that this model overfit the data with a difference between models of 10.9%. The RCC for human still exceeded 75% (77.5%). The two lowest classification results came from horse with an RCC of 69.6% and other-avian (72.1%). The RCCs for the other animal categories were all above 75% (Table 20).

Table 20 Discriminant analysis of known source enterococci isolates from Oso Creek library. Seven-way model—human vs. cow vs. horse vs. dog vs. seagull vs. "other avian" vs. wildlife (non-avian) (equal prior probabilities)

Classification Results

			Predicted Group Membership							
		Type	Human	Cow	Horse	Dog	Seagull	Other Avian	Non-Avian Wildlife	Total
Original	Count	Human	86	3	3	3	0	14	2	111
		Cow	5	122	6	1	0	8	9	151
		Horse	5	7	64	2	0	6	8	92
		Dog	1	3	6	138	11	8	2	169
		Seagull	0	3	0	9	90	0	1	103
		Other Avian	9	7	23	4	0	199	34	276
		Non-Avian Wildlife	4	6	16	2	0	14	141	183
	%	Human	**77.5**	2.7	2.7	2.7	.0	12.6	1.8	100.0
		Cow	3.3	**80.8**	4.0	.7	.0	5.3	6.0	100.0
		Horse	5.4	7.6	**69.6**	2.2	.0	6.5	8.7	100.0
		Dog	.6	1.8	3.6	**81.7**	6.5	4.7	1.2	100.0
		Seagull	.0	2.9	.0	8.7	**87.4**	.0	1.0	100.0
		Other Avian	3.3	2.5	8.3	1.4	.0	**72.1**	12.3	100.0
		Non-Avian Wildlife	2.2	3.3	8.7	1.1	.0	7.7	**77.0**	100.0

a. 77.4% of original grouped cases correctly classified.

b. 66.5% of cross-validated grouped cases correctly classified.

For each model, the ARCCs decreased as the models increased in complexity with more animal categories and all but the two-way model, overfit according to EPA standards. Noteably, only one animal source (horse at 69.6%) in only one model (seven-way) was below 70% correct classification, with the majority of classification models discriminating animal sources at above 80%.

Stockel and Harwood (2007) articulate that the probability of being correct by random chance alone increases as the number of classification categories/models decreases. They contend that a benefit over random should be used to evaluate the accuracy of ARCCs. In view of the fact that random classification in a seven-way model would provide RCCs of 14.3% and that the lowest correct classification in this study was 69.6%, the benefit over random would noteably be 55.3% in the most complex model.

Random Forests Modeling of the Library

The overall and categorical ranges of ARCCs and RCCs for individual classes for each model are outlined in Table 21. Since Random Forests applies a "random" element to choosing particular variables from discriminants, it has a slightly different error rate than LDA, which has a unique ARCC that does not change with each run. For RF, ARCCs are derived by subtracting the out-of-bag (OOB) error rate from 100% for each run with the same method applying for each rate of correct classification (RCC) per animal class.

In the case of Random Forests, the bootstrap sample is a random selection of known source bacterial isolates, chosen with replacement. The default size of the bootstrap sample is the same size as the isolate library. Since the RF samples are chosen with replacement, some isolates are randomly included more than once, while others are omitted. The OOB is approximately one-third of cases in a dataset that are excluded from the sample set when building each tree (the isolates not included for a given bootstrap sample).

Random Forests does take into account sample sizes and since the human category has 72 profiles versus the non-human category containing 974, this distribution has a detrimental effect on RCC rates for the sources with lower samples sizes, particularly human. In order to counteract this, RF has a stratification option that allows the user to define how many random isolates per source class are used in constructing the forest. Since there is a random element to each RF run, each of the five models was run 100 times and RCC and ARCC ranges were recorded for each run and reported next to the average RCC and ARCC of the 100 RF runs (Table 21).

The highest ARCC of 78.3% was for the two-way model. The RCC for human was 79.6% with a non-human RCC of 78.2%. The ARCC for the three-way model was reduced by 5.9% to an ARCC of 72.4% in comparison to the two-way classification model. The RCC for human remained above 70% RCC, and the RCC for domestic animal was higher than that for wild animal. The four- and five-way models were similar in RCC and ARCC rates, with the exception of dog, for which the RCC was reduced 12.6%, and seagull for which the RCC was 83.5%. The seven-way classification model had the lowest overall ARCC at 66.5%. Groups had a spread of RCCs with the lowest being “other-avian” at 56.6% and the highest being seagull at 84.0%. The remaining five categories had similar RCCs, around the overall ARCC (Table 21).

Table 21 Random Forests analysis of the known source *Enterococcus* isolates in the Oso Creek library

Random Forest - Stratified		RCC and RCC Ranges (standard error) (%) for Each Class	ARCC and ARCC Ranges (standard error) (%) for Overall Models
2-Way Classification	Human	79.6 ± 1.8	78.3 ± 1.3
	Non-Human	78.2 ± 0.8	
3-Way Classification	Human	73.5 ± 1.6	72.4 ± 1.0
	Domestic Animal	76.1 ± 0.9	
	Wild Animal	69.8 ± 0.5	
4-Way Classification	Human	71.6 ± 2.0	72.5 ± 0.7
	Livestock	74.7 ± 0.1	
	Dog	82.0 ± 0.1	
	Wild Animal	69.9 ± 0.6	
5-Way Classification	Human	72.1 ± 1.8	72.4 ± 1.2
	Livestock	75.6 ± 1.0	
	Dog	69.4 ± 1.4	
	Seagull	83.5 ± 1.1	
	Wildlife (Avian/Non)	70.9 ± 0.7	
7-Way Classification	Human	64.0 ± 2.0	66.5 ± 1.6
	Cow	72.9 ± 1.4	
	Horse	65.0 ± 2.4	
	Dog	68.6 ± 1.5	
	Seagull	84.0 ± 1.3	
	Other Avian	56.6 ± 1.4	
	Non-Avian Wildlife	72.4 ± 1.3	

Validation of Library

Validation of Discriminant Analysis

For each LDA classification, a cross-validation (jackknife) analysis was performed to ensure the representativeness of the library. Cross-validation is a function of SPSS®, which removes one isolate at a time and classifies it based on the remaining isolates using LDA. According to the US EPA, if the difference

in ARCC is less than 5% between the original LDA and the cross-validated LDA, the library can be considered representative (US EPA 2005). Results of ARCC values for original LDAs for all five models, as well as the ARCC values and differences for the cross-validated LDA are shown in Table 22.

As shown in Table 22, four of the five models did not fall within the US EPA recommendation of a 5% difference in ARCC between original LDA and cross-validated LDA models for representativeness. The only model that was within the 5% difference threshold was the two-way classification model. The three-, four-, and five-way classification models were slightly above the 5% threshold. The seven-way classification model was more than double the threshold and thus can be considered to overfit the model. This, however, is to be expected as model complexity increases with the addition of animal groups (Hagedorn *et al*. 2003; Moussa and Massengale 2008).

Table 22 Comparison between original and cross-validated ARCCs for the five models used in the study

	ARCC (%) for Linear Discriminant Analysis	**ARCC (%) for Cross Validation**	**Difference Between Original and Cross Validated ARCCs (%)**
2-Way Classification	92.5	89.6	2.9
3-Way Classification	81.5	75.8	5.7
4-Way Classification	81.2	73.3	7.9
5-Way Classification	81.8	73.5	8.3
7-Way Classification	77.4	66.5	10.9

Additional testing was performed on the LDA models using R statistical software, by challenging the library and each of the five models using training and test sets. To challenge the library, 20% (217 isolates) of the known source profiles were randomly excluded from the library and analyzed as unknowns with LDA. Ideally, classification of this randomly selected 20% (test set) should occur at a rate similar to the original analysis. This challenge was repeated 100 times and the averages of the ARCCs for each of the five models test sets is listed in comparison to the original training set (all 1085 isolates) in Table 23.

Table 23 Summary of mean ARCCs for training and test set data for LDA of known source isolates

Linear Discriminant Analysis	ARCC (%)	
	Training Set	Test Set
2-Way Classification	92.5	85.2
3-Way Classification	81.5	73.3
4-Way Classification	81.2	72.3
5-Way Classification	81.8	74.8
7-Way Classification	77.4	65.6

In two- through five-way classification models, the test sets performed with a ~7-9% difference in ARCC. However, for the seven-way classification, the test set differed in comparison to the training set by 11.8% (Table 23).

Validation of Random Forests

Jackknife cross-validation is not applicable for Random Forests analyses, since their ARCC's are already reported only for those isolates not included in

the construction of each tree. However, the same validation challenge test that was applied to LDA was also applied to RF. Table 24 summarizes the ARCCs from the training and test sets for RF stratification classification models.

Table 24 Summary of mean ARCCs for training and test set data for RF of known source isolates

Random Forests – Stratified	ARCC (%)	
	Training Set	Test Set
2-Way Classification	78.3	73.7
3-Way Classification	72.4	70.5
4-Way Classification	72.5	70.5
5-Way Classification	72.4	71.9
7-Way Classification	66.5	64.6

The RF-stratified training and test sets performed more closely to each other than the LDA training and test sets (Tables 23 and 24). The greatest difference between the training and test set for RF-stratified was 4.6%, for the two-way classification model. The smallest difference was the five-way classification model with a 0.5% difference (Table 24). It should be noted that due to the differences in construction, it is difficult to adequately compare LDA and RF classification models.

Validation of the Oso Creek Library via Analysis of Enterococci Isolated Outside the Oso Watershed

In order to test the geographical and temporal stability of the library, human and cow enterococcal isolates from outside the Oso watershed, isolated during 2003 by Stewart, were tested as unknowns. Tables 25 and 26 summarize the findings using LDA and RF-stratified to classify the human and cow "unknown" test profiles. Human volunteer and portable toilet enterococci were isolated from Padre Island, Texas in June and July of 2003. Cow enterococci were isolated from Rockport, Texas and Annaville, Texas in June of 2003 (Stewart 2005). Neither LDA nor RF were able to correctly classify unknown cow and human test isolates with a high rate of correct classification (Table 25 and 26).

Table 25 Summary of LDA and RF-stratified classification of human source "unknown" enterococci isolated from outside the Oso watershed using the Oso Creek library.

Human Unknown Isolates		LDA Isolate Classification (%)	RF-stratified Isolate Classification (%)
2-Way Classification	Human	18.8	3.1
	Non-Human	81.3	96.9
3-Way Classification	Human	12.5	2.1
	Domestic Animal	40.6	38.5
	Wild Animal	46.9	59.3
4-Way Classification	Human	7.3	2.1
	Livestock	8.3	13.5
	Dog	45.8	47.9
	Wild Animal	38.5	36.5
5-Way Classification	Human	2.1	4.2
	Livestock	9.4	12.5
	Dog	34.4	18.8
	Seagull	52.1	63.5
	Wildlife (Avian/Non)	2.1	1.0
7-Way Classification	Human	1.0	4.2
	Cow	4.2	3.1
	Horse	3.1	10.4
	Dog	32.3	16.7
	Seagull	54.2	62.5
	Other Avian	1.0	0.0
	Non-Avian Wildlife	4.2	3.1

Table 26 Summary of LDA and RF-stratified classification of cow source "unknown" enterococci isolated from outside the Oso watershed using the Oso Creek library.

Cow Unknown Isolates		LDA Isolate Classification (%)	RF-stratified Isolate Classification (%)
2-Way Classification	Human	20.0	4.3
	Non-Human	80.0	95.7
3-Way Classification	Human	15.7	0.0
	Domestic Animal	62.9	88.6
	Wild Animal	21.4	11.4
4-Way Classification	Human	10.0	4.3
	Livestock	12.9	31.4
	Dog	55.7	50.0
	Wild Animal	21.4	14.3
5-Way Classification	Human	8.6	2.9
	Livestock	8.6	31.4
	Dog	55.7	50.0
	Seagull	22.9	14.3
	Wildlife (Avian/Non)	4.3	1.4
7-Way Classification	Human	7.1	2.9
	Cow	11.4	7.1
	Horse	1.4	28.6
	Dog	52.9	45.7
	Seagull	21.4	12.9
	Other Avian	4.3	0.0
	Non-Avian Wildlife	1.4	2.9

Comparison of LDA and Random Forest Models

Table 27 shows a comparison of ARCCs and RCCs achieved using LDA and RF-stratification. Overall, ARCCs using LDA were 8.7% to 14.2% higher than those using RF-stratification. Both LDA and RF ARCCs were inversely related to model complexity. As model complexity increased with more categories, ARCCs decreased. This was mostly true for RCCs as well, except in

a few cases such as livestock in four- and five-way classification models, as well as seagull in five- and seven-way classification models.

Table 27 Summary of ARCCs and RCCs using LDA and RF-stratification for five selected classification models for the known source library

		RCCs for LDA (%)	ARCCs for LDA (%)	RCCs for RF-Stratified (%)	ARCCs for RF-Stratified (%)
2-Way Classification	Human	85.6	92.5	79.6	78.3
	Non-Human	93.3		78.2	
3-Way Classification	Human	83.8	81.5	73.5	72.4
	Domestic Animal	79.1		76.1	
	Wild Animal	82.7		69.8	
4-Way Classification	Human	79.3	81.2	71.6	72.5
	Livestock	78.3		74.7	
	Dog	88.8		82.0	
	Wild Animal	80.6		69.9	
5-Way Classification	Human	79.3	81.1	72.1	72.4
	Livestock	79.0		75.6	
	Dog	82.2		69.4	
	Seagull	87.4		83.5	
	Wildlife(Avian/Non)	82.6		70.9	
7-Way Classification	Human	77.5	77.4	64.0	66.5
	Cow	80.8		72.9	
	Horse	69.6		65.0	
	Dog	81.7		68.6	
	Seagull	87.4		84.0	
	Other Avian	72.1		56.6	
	Non-Avian Wildlife	77.0		72.4	

The highest ARCC was achieved by two-way classification using LDA (93.3%). The lowest ARCC was the “other avian” animal category in the seven-way classification model using RF-stratified (56.6%) (Table 27). Overall, analyses using LDA achieved higher ARCCs than RF for all five classification models.

Classification of Unknown Source Isolates

Table 11 summarizes the composition of the unknown source isolate database. A total of 792 Oso Creek water and sediment isolates were profiled via CSU and ARP. These composite profiles were then compared to the Oso Creek known source animal library using both LDA and RF.

Using LDA for two-way classification (human vs. non-human), 8.2% of the unknown source creek and sediment isolates were categorized as human source and 91.8% as non-human source (727 of the 792 isolates falling into non-human predicted group membership) (Table 28). Further discrimination involved utilizing additional models that divided the non-human group into various animal groups. The three-way model (human vs. domestic animal (cow, horse, and dog) vs. wild animal (avian and non-avian wildlife) classified 6.7% of the creek isolates as human source, while 29.7% of the isolates were classified as domestic animal and the majority (63.6%) as wild animal (avian/non) (Table 29). The four-way model further divided the domestic animal group into livestock and dog. This model similarly classified 6.4% of the isolates as human and 31.6% as livestock with 4.9% as dog, and the majority (57.1%) as wild animal sources (avian/non) (Table 30).

Table 28 Discriminant analysis of unknown source enterococci isolates compared to Oso Creek library. Two-way model—human vs. non-human (equal prior probabilities)

Classification Results

			Predicted Group Membership		
		Type	Human	Non-Human	Total
Original	Count	Human	95	16	111
		Non-Human	65	909	974
		Unknowns	65	727	792
	%	Human	85.6	14.4	100.0
		Non-Human	6.7	93.3	100.0
		Unknowns	**8.2**	**91.8**	100.0

a. 92.5% of original grouped cases correctly classified.

Table 29 Discriminant analysis of unknown source enterococci isolates compared to Oso Creek library. Three-way model—human vs. domestic animal (cow/horse/dog) vs. wild animal including bird (equal prior probabilities)

Classification Results

			Predicted Group Membership			
		Type	Human	Domestic	Wild Animal	Total
Original	Count	Human	93	6	12	111
		Domestic Animal	26	326	60	412
		Wild Animal	17	80	465	562
		Unknowns	53	235	504	792
	%	Human	83.8	5.4	10.8	100.0
		Domestic Animal	6.3	79.1	14.6	100.0
		Wild Animal	3.0	14.2	82.7	100.0
		Unknowns	**6.7**	**29.7**	**63.6**	100.0

a. 81.5% of original grouped cases correctly classified.

Table 30 Discriminant analysis of unknown source enterococci isolates compared to Oso Creek library. Four-way model—human vs. livestock vs. dog vs. wild animal (equal prior probabilities)

Classification Results

			Predicted Group Membership				
		Type	Human	Livestock	Dog	Wild Animal	Total
Original	Count	Human	88	6	4	13	111
		Livestock	18	190	9	26	243
		Dog	0	5	150	14	169
		Wild Animal	17	66	26	453	562
		Unknowns	51	250	39	452	792
	%	Human	79.3	5.4	3.6	11.7	100.0
		Livestock	7.4	78.2	3.7	10.7	100.0
		Dog	.0	3.0	88.8	8.3	100.0
		Wild Animal	3.0	11.7	4.6	80.6	100.0
		Unknowns	**6.4**	**31.6**	**4.9**	**57.1**	100.0

a. 81.2% of original grouped cases correctly classified.

A five-way model separated seagull from wildlife and showed very similar percentages for each group as the four-way model with less than 1% of the isolates classifying as seagull (Table 31).

Table 31 Discriminant analysis of unknown source enterococci isolates compared to Oso Creek library. Five-way model—human vs. livestock vs. dog vs. seagull vs. wildlife (avian/non) (equal prior probabilities)

Classification Results

			Predicted Group Membership					
		Type	Human	Livestock	Dog	Seagull	Wildlife (Avian/Non)	Total
Original	Count	Human	88	8	3	0	12	111
		Livestock	16	192	9	0	26	243
		Dog	3	6	139	11	10	169
		Seagull	0	2	10	90	1	103
		Wildlife (Avian/Non)	16	57	7	0	379	459
		Unknowns	53	249	30	5	455	792
	%	Human	79.3	7.2	2.7	.0	10.8	100.0
		Livestock	6.6	79.0	3.7	.0	10.7	100.0
		Dog	1.8	3.6	82.2	6.5	5.9	100.0
		Seagull	.0	1.9	9.7	87.4	1.0	100.0
		Wildlife (Avian/Non)	3.5	12.4	1.5	.0	82.6	100.0
		Unknowns	**6.7**	**31.4**	**3.8**	**.6**	**57.4**	100.0

a. 81.8% of original grouped cases correctly classified.

The most discriminatory model developed was the seven-way classification which further split the livestock group into cow and horse, as well as splitting wildlife into both “other avian” (no seagull) and non-avian wildlife (mammals). This model predicted that 13.4% and 18.8% of the isolates were from cow and horse, respectively with <5% each from dog, human, and seagull and the majority of isolates (59.6%) as “other avian” species and non-avian wildlife (30.6% and 29.0%, respectively) (Table 32).

Table 32 Discriminant analysis of unknown source enterococci isolates compared to Oso Creek library. Seven-way model—human vs. cow vs. horse vs. dog vs. seagull vs. "other avian" vs. wildlife (non-avian) (equal prior probabilities)

Classification Results

			Predicted Group Membership							
		Type	Human	Cow	Horse	Dog	Seagull	Other Avian	Non-Avian Wildlife	Total
Original	Count	Human	86	3	3	3	0	14	2	111
		Cow	5	122	6	1	0	8	9	151
		Horse	5	7	64	2	0	6	8	92
		Dog	1	3	6	138	11	8	2	169
		Seagull	0	3	0	9	90	0	1	103
		Other Avian	9	7	23	4	0	199	34	276
		Non-Avian Wildlife	4	6	16	2	0	14	141	183
		Unknowns	35	106	149	27	3	242	230	792
	%	Human	77.5	2.7	2.7	2.7	.0	12.6	1.8	100.0
		Cow	3.3	80.8	4.0	.7	.0	5.3	6.0	100.0
		Horse	5.4	7.6	69.6	2.2	.0	6.5	8.7	100.0
		Dog	.6	1.8	3.6	81.7	6.5	4.7	1.2	100.0
		Seagull	.0	2.9	.0	8.7	87.4	.0	1.0	100.0
		Other Avian	3.3	2.5	8.3	1.4	.0	72.1	12.3	100.0
		Non-Avian Wildlife	2.2	3.3	8.7	1.1	.0	7.7	77.0	100.0
		Unknowns	**4.4**	**13.4**	**18.8**	**3.4**	**.4**	**30.6**	**29.0**	100.0

a. 77.4% of original grouped cases correctly classified.

Stratified RF was also utilized to classify unknown source isolates using two- through seven-way classification models. A summary of both RF and LDA results are shown in Table 33. In the two- through five-way classification models, results differed between the two analyses, mostly in the proportions of isolates

classified into the human category. However, LDA and RF results were similar for the seven-way model. In the two-way classification, LDA and RF-stratified differ in the human and non-human categories by 28.5%, with LDA classifying 8.2% of the isolates as human vs. RF classifying 36.7% in the category. In three-way classification models, individual animal classes differed between 3.9% and 28.9%. The proportion of isolates classifying into the human category using the RF-stratified method decreased with increasing complexity of the model from a high in the two-way of 36.7% to 31.7% in the three-way and further to 3.3% in the seven-way model. The largest discrepancy of 28.9% between results using the two analyses was in the three-way classification model for the wild animal category, with an LDA unknown classification of 63.6% and an RF classification of 34.7%, primarily due to the much higher percent classifying as human with the latter method. Overall, both statistical methods identified “other avian” and non-avian wildlife as the source of the majority of unknown source isolates and human source as a very minor proportion (Table 33).

Table 33 Summary of LDA and RF-stratified classification of unknown source isolates

		Classification of Unknown Source Isolates (%)	
		LDA	**RF-Stratified**
2-Way Classification	Human	8.2	36.7
	Non-Human	91.8	63.3
3-Way Classification	Human	6.7	31.7
	Domestic Animal	29.7	33.6
	Wild Animal	63.6	34.7
4-Way Classification	Human	6.4	23.1
	Livestock	31.6	25.5
	Dog	4.9	4.3
	Wild Animal	57.1	47.1
5-Way Classification	Human	6.7	20.8
	Livestock	31.4	26.3
	Dog	3.8	4.7
	Seagull	0.6	0.0
	Wildlife (Avian/Non)	57.4	48.2
7-Way Classification	Human	4.4	3.3
	Cow	13.4	3.9
	Horse	18.8	19.1
	Dog	3.4	3.7
	Seagull	0.4	0.0
	Other Avian	30.6	43.4
	Wildlife (Non-Avian)	29.0	26.8

Dry and Wet Events

Tables 34 and 35 summarize the LDA and RF-stratified source classifications of creek water and sediments enterococci isolates from wet and dry events for two- and seven-way classification models. SPSS® tables from LDA for unknown creek, water and sediments, as well as wet and dry events are shown in Appendix F.

Comparisons between wet (following rainfall) and dry sampling events showed similar results for unknown isolate classification, except for “other avian” and non-avian wildlife. The majority of isolates classified as “other avian” (*i.e.* birds other than seagull), and non-avian wildlife (~60% isolates) during both wet and dry periods within Oso Creek water and sediments, with about 30% classifying as livestock. However, during dry weather ~39% of the isolates classified as avian (other than seagull) with ~20% as wildlife, but following rainfall this arrangement was reversed and ~39% were classified as non-avian wildlife and ~20% as avian. The proportion of isolates classifying as human was <10% under either condition (Table 34).

Table 34 Classification of unknown source Oso Creek water and sediment isolates collected in dry weather and following rainfall using LDA

Linear Discriminant Analysis		Wet Events (% isolates)	Dry Events (% isolates)	Both Events Combined (% isolates)
2-Way Classification	Human	7.9	8.5	8.2
	Non-Human	92.1	91.5	91.8
7-Way Classification	Human	4.9	4.0	4.4
	Cow	13.1	13.6	13.4
	Horse	19.6	18.1	18.8
	Dog	2.7	4.0	3.4
	Seagull	0.3	0.5	0.4
	Other Avian	20.4	39.3	30.6
	Non-Avian Wildlife	39.0	20.5	29.0

Using RF-stratified analysis, a much higher proportion of isolates were classified as human (~39%) in the two-way classification model, but as with LDA the proportion was similar for both dry and wet events (Table 35). The proportion

of isolates classifying as human was much lower in the seven-way classification model – 6.0% during wet events and 2.8% during dry events. Similarly to the LDA results for wet events, non-avian wildlife was the largest category (40.1%), followed by "other avian" and horse (26.4% and 18.3% isolates, respectively) and as with LDA, the converse was found during dry events, with less than 20% of the isolates classified as non-avian wildlife, while "other avian" accounted for almost three-fifths of the enterococci (Table 35). Minimal numbers of isolates classified as human, cow, or dog for either wet or dry events, and no isolates classified as seagull using RF-stratified (Table 35).

Table 35 Classification of unknown source Oso Creek water and sediment isolates collected in dry weather and following rainfall using RF-stratified

Random Forest – Stratified		Wet Events (% isolates)	Dry Events (% isolates)	Both Events Combined (% isolates)
2-Way Classification	Human	39.8	39.3	36.7
	Non-Human	60.2	60.7	63.3
7-Way Classification	Human	6.0	2.8	3.3
	Cow	6.0	1.9	3.9
	Horse	18.3	13.6	19.1
	Dog	3.3	4.5	3.7
	Seagull	0.0	0.0	0.0
	Other Avian	26.4	59.1	43.4
	Non-Avian Wildlife	40.1	18.1	26.8

Seven-way classification models for both LDA and RF-stratified showed that both "other avian" and non-avian wildlife are the primary contributors of enterococci in Oso Creek water and sediments during both dry and wet events. Both statistical analyses also demonstrated the same inversely proportional trend between "other avian" and non-avian wildlife during wet and dry events, with non-

avian higher during wet events and lower in dry, and “other avian” lower in wet and higher in dry (Tables 34 and 35). Horse classification was similar in both analyses but the proportion of isolates classifying as cow, was much lower in RF-stratified in comparison to the LDA seven-way model. A noted difference between the two statistical analyses is that in two-way classification models, RF-stratified, the human category was almost 40% of the isolates but was greatly reduced in seven-way modeling. Conversely, LDA showed a consistently low proportion of human isolates in all models.

West Oso Creek vs. Oso Creek: Dry Events

Tables 36 and 37 summarize the LDA and RF-stratified classifications of unknown source water and sediments enterococci isolates from the secondary tributary, West Oso Creek, and upper Oso Creek during dry events using two- and seven-way classification models. SPSS® tables from LDA for both West Oso Creek and Oso Creek during dry events are shown in Appendix F.

Dry weather results using LDA were similar for both West Oso Creek and Oso Creek, with the largest proportion of isolates classifying as “other avian.” RF-stratification results showed the discrepancy in the proportion of isolates classifying as human in two-way vs. seven-way modeling (Table 37). In the stratified RF seven-way classification, almost three-fourths of the enterococcal isolates in West Oso Creek were “other avian” during dry events with very few classifying as human, cow, or seagull. In the stations on the main portion of Oso Creek, 57.4% of the unknowns also classified as “other avian” (Table 37).

Table 36 Classification of water and sediment isolates from West Oso Creek and main Oso Creek for dry weather events, using LDA

Linear Discriminant Analysis on Dry Events		West Oso Creek (% isolates)	Main Oso Creek (% isolates)	All of Oso Creek (% isolates)
		Stations 18501	*Stations 18499, 18500, & 20559*	*All Stations*
2-Way Classification	Human	8.0	8.6	8.5
	Non-Human	92.0	91.4	91.5
7-Way Classification	Human	5.3	3.7	4.0
	Cow	16.0	13.1	13.6
	Horse	18.7	18.0	18.1
	Dog	5.3	3.7	4.0
	Seagull	0.0	0.6	0.5
	Other Avian	36.0	40.0	39.3
	Non-Avian Wildlife	18.7	20.9	20.5

Table 37 Classification of water and sediment isolates from West Oso Creek and main Oso Creek for dry weather events, using RF-stratified

Random Forest - Stratified on Dry Events		West Oso Creek (% isolates)	Main Oso Creek (% isolates)	All of Oso Creek (% isolates)
		Stations 18501	*Stations 18499, 18500, & 20559*	*All Stations*
2-Way Classification	Human	41.3	31.7	39.3
	Non-Human	58.7	68.3	60.7
7-Way Classification	Human	1.3	2.0	2.8
	Cow	1.3	2.9	1.9
	Horse	9.3	14.0	13.6
	Dog	1.3	4.9	4.5
	Seagull	0.0	0.0	0.0
	Other Avian	72.0	57.4	59.1
	Non-Avian Wildlife	14.8	18.9	18.1

Thus, during dry events, both LDA and RF-stratified analyses showed that "other avian"— *i.e.* birds other than seagull were the largest contributors of enterococci to both West Oso Creek and Oso Creek, followed by non-avian wildlife and horse, to a lesser extent. RF-stratification again categorized a much higher proportion as human in two-way models as compared to LDA.

West Oso Creek vs. Oso Creek: Wet Events

Tables 38 and 39 summarize the LDA and RF-stratified classifications of enterococci isolates from creek water and sediments from both the secondary tributary, West Oso Creek and Oso Creek following rainfall, using two- and seven-way classification models. SPSS® tables from LDA for both West Oso Creek and Oso Creek during wet events are shown in Appendix F.

During wet events, similar results were seen for both sections of the creek using LDA. The majority of the isolates classified as cow, horse, "other avian," and non-avian wildlife with few isolates classifying as human, dog, and seagull (Table 38). The highest proportion of isolates was categorized as non-avian wildlife with 45.1% in West Oso Creek and 32.0% in Oso Creek (Table 38).

Table 38 Classification of water and sediment isolates from West Oso Creek and main Oso Creek following rainfall, using LDA

Linear Discriminant Analysis on Wet Events		West Oso Creek (% isolates) *Stations 18501 & 20198*	Main Oso Creek (% isolates) *Stations 18499, 18500, & 20559*	All of Oso Creek (% isolates) *All Stations*
2-Way Classification	Human	7.7	8.1	7.9
	Non-Human	92.3	91.9	92.1
7-Way Classification	Human	4.6	5.2	4.9
	Cow	12.3	14.0	13.1
	Horse	22.6	16.3	19.6
	Dog	0.5	5.2	2.7
	Seagull	0.0	0.6	0.3
	Other Avian	14.9	26.7	20.4
	Non-Avian Wildlife	45.1	32.0	39.0

Using RF-stratification the same pattern of high percentages of isolates classifying as human in the two-way classification with much lower in the seven-way classification model was seen (Table 39). Most isolates were classified as horse, “other avian,” and non-avian wildlife with few isolates classifying as human, cow, dog, or seagull (Table 39). However, with RF a difference was seen between the two parts of the creek during wet events: the highest contributor of enterococci in the main section of the creek was “other avian” sources (38.4%); conversely, in West Oso Creek the highest contributor of enterococci was non-avian wildlife with the majority of isolates classifying under this category (52.8%) (Table 39).

Table 39 Classification of water and sediment isolates from West Oso Creek and main Oso Creek following rainfall, using RF-stratified

Random Forest - Stratified on Wet Events		West Oso Creek (% isolates)	Main Oso Creek (% isolates)	All of Oso Creek (% isolates)
		Stations 18501 & 20198	*Stations 18499, 18500, & 20559*	*All Stations*
2-Way Classification	Human	44.1	33.1	39.8
	Non-Human	55.9	66.9	60.2
7-Way Classification	Human	5.1	7.0	6.0
	Cow	4.6	7.6	6.0
	Horse	21.0	13.4	18.3
	Dog	0.0	5.8	3.3
	Seagull	0.0	0.0	0.0
	Other Avian	16.4	38.4	26.4
	Non-Avian Wildlife	52.8	27.9	40.1

The two methods provided similar results for the two sections of the creek in wet weather conditions, using seven-way classification except for cow, “other avian,” and non-avian wildlife categories. Isolates classified as cow were far fewer using RF-stratification than LDA. In the main section of Oso Creek, RF indicated that “other avian” was the primary contributor (38.4% of the isolates) while LDA indicated “other avian” and non-avian wildlife were the primary contributors. However, LDA and RF both had similar results for West Oso Creek in terms of “other avian” and non-avian wildlife (Tables 38 and 39).

DISCUSSION

In this microbial source tracking study, utilizing *Enterococcus* with a toolbox approach in both laboratory methods and statistical analysis proved an effective technique for discriminating animal fecal sources. Animal fecal sampling was primarily carried out along the upper portions of Oso Creek but in order to obtain a representative sampling of the animal population, fecal samples were collected at multiple locations within the Oso watershed. Using the microbial identification system and Kirby-Bauer disk diffusion assays, carbon source utilization and antibiotic resistance profiles were generated for each *Enterococcus* isolate. Carbon source utilization served two purposes: first to identify and speciate animal and creek enterococci, and second to provide profiles of enterococci carbon usage for source tracking.

Speciation provided information about the enterococcal flora of the sampled animal species. *E. faecalis* was the most prevalent species in animal fecal samples, similar to findings in previous studies (Godfree *et al*. 1997; Facklam *et al*. 2002; Meschke and Boyle 2007; Carrero-Colón *et al*. 2011). The second most frequently identified species from animal sources was *E. casseliflavus.* This species of *Enterococcus* has been found in horse feces, but has also been frequently isolated from the surface of plants (Ulrich and Müller 1998). Aarestrup *et al*. (2002) found *E. casseliflavus* was a transient species in the intestines of animals. In this study, *E. casseliflavus* was isolated from all animal fecal sources sampled with the exception of dog,

with the highest numbers isolated from cow, sheep (data not included in Oso Creek library, see Appendix E), horse, bird, and human.

Animal species containing *E. casseliflavus* in their gut tend to be herbivorous, though *E. casseliflavus* has been found in other animals (Krause and Khafipour 2011). *E. casseliflavus* was the most abundant *Enterococcus* species in cow fecal matter and wastewater in this project (48.31% and 21.84%). This contrasts with a previous study by Stewart (2005), in which *E. mundtii* was determined to be predominant in cow (43.2%), followed by *E. casseliflavus* (20.5%). Additionally, Stewart's study also found *E. casseliflavus* in human samples, but constituting only 6.7% of isolated enterococci versus *E. faecalis* that constituted 62.5% (Stewart 2005). Other studies have provided mixed results. For example, Devrise *et al*. (1987) found *E. casseliflavus* absent from cow or sheep, while Petersson-Wolf *et al*. (2008) detected *E. casseliflavus* in cattle (28.4%), and Thal *et al*. (1995) detected *E. casseliflavus* in horse. Overall, the presence of *E. casseliflavus* appears to be inconsistent in animals and more research is needed to understand its colonization in certain animal hosts.

This study's results agreed with Franz *et al*., who found lower levels of *E. faecium* and *E. faecalis* in livestock than human sources (Franz *et al*. 1999). Godfree *et al*. isolated *E. hirae* only from human fecal material (Godree *et al. 1997)* in contrast to this study where *E. hirae* was not isolated from human sources and was only infrequently found in bird, dog, cow, horse, and wildlife (all <2%) (Table 10).

Species-specific enterococci were only isolated in very low numbers, with *E. pseudoavium* and *E. malodoratus* being exclusively isolated from wastewater efffluent. *E. pseudoavium* is a pathogen causing bovine mastitis (Aarestrup *et al*. 2002). *E. pseudoavium* and *E. malodoratus* are also associated with swine feces, pork carcasses, and foods such as fresh and spoiled sausage (Devriese *et al*. 1994; Klein 2003). *E. solitarius* was isolated from only horse samples. Ennahar and Cai (2005), based on previous studies, as well as biochemical and genetic evidence, suggested that *E. solitarius* should be transferred to the genus *Tetragenococcus* (Facklam *et al*. 2002; Klein 2003). Since the MicroLog™ Microbial Identification System Release 4.20.04 is a 2004 Biolog bacterial reference library, the re-naming of *E. solitarius* to the new species *Tetragenococcus solitarius* in 2005, was not incorporated into the system (Biolog 2004; Ennahar and Cai 2005). Therefore, *E. solitarius* is not counted as a species-specific *Enterococcus* for the purposes of this study.

Enterococcus faecium, a species of the *Enterococcus* genus generally associated with human fecal matter (Wheeler *et al*. 2002; Scott *et al*. 2005; Ogier and Serror 2008), was isolated from bird, cow, horse, and wildlife fecal samples. Dog samples contained the highest percentage (22.4%) of *E. faecium* in this study, in contrast to a previous study by Stewart where *E. faecium* only accounted for 10.8% of dog *Enterococcus* isolates (Stewart 2005). *E. faecalis* accounted for 20.7% of effluent and influent isolates in the current study, whereas previous studies report 80-95% enterococci of this

species in human feces (Hagedorn *et al*. 2003; Carrero-Colón *et al*. 2011). This difference might be due to survival during the treatment of wastewater in primary influent treatment plants and suggests that *E. casseliflavus* and *E. gallinarum* might be more adapted to surviving the treatment process given their levels in treated wastewater (Table 10; Appendix E).

Enterococci are divided into five functional groups based on their abilities to ferment certain sugars and hydrolyze certain amino acids (Facklam *et al*. 2002). *E. casseliflavus* and *E. gallinarum* belong to the second-class functional group, which can utilize the largest array of sugars and amino acids in comparison to the other four functional groups (Facklam *et al*. 2002). Potentially, this might allow for members of this group to survive in a greater range of environments with different available nutrients. *E. casseliflavus* and *E. gallinarum* are also both motile species, a rare trait among enterococci (Collins *et al*. 1986; Facklam *et al*. 2002). This ability, coupled with *E. cassliflavus* and *E. gallinarum*'s intrinsic resistance to glycopeptides via the built-in *vanC* gene, could explain how these two species survive treatment processing and any residual glycopeptides in wastewater, which can be highly fatal for vancomycin susceptible enterococci. Although a few *E. faecalis* were isolated from wastewater, high percentages of this species (as a proportion of total isolates) were recovered from fecal samples from both wildlife and birds.

In order to assess sources of contamination in the Oso Creek watershed, water and sediment *Enterococcus* isolates were profiled using

both CSU and ARP for comparisons with known animal isolate CUPs and ARPs. Speciation via the MicroLog™ Microbial Identification System showed that upper Oso Creek water and sediments contained a large percentage of both of *E. mundtii* (45.41%) and *E. faecalis* (29.19%). *E. mundtii* is a non-motile, yellow-pigmented enterococcal strain that is strongly associated with plants, such as grasses and vegetables, and has also been isolated from soil, water, fish, and crustaceae (Collins *et al*. 1986; Leclerc *et al*. 1996; Ulrich and Müller 1998; Aarestrup *et al*. 2002; Klein 2003). It is unclear whether *E. mundtii* is a naturally occurring bacterium in these environments or if it is an environmental contaminant stemming from other sources, such as fecal matter.

Enterococcus mundtii was isolated from all sampled animal sources in this project and constituted ~9% of total recovered enterococci (Table 8), in comparison with ~20% of all enterococci isolated by Stewart in a previous project, which was limited to bird, cow, human, and dog sources (Stewart 2005). The reason for this disproportionate percentage of enterococci being deposited by animals versus the enterococcal species that are being isolated from within the creek is largely unknown; yet, there are several possibilities. Studies have shown that populations in secondary habitats can differ significantly from those in primary habitats from which the organisms originated (Gordon *et al.* 2002). Survivability studies have demonstrated that enterococci have reproductive capability under non-extreme environments with survival rates similar to waterborne bacterial pathogens (Lleo *et al*. 2005).

However, survival rates are also dependent on other factors such as predation, nutrient availability, antimicrobials, microbial competition, and environmental, chemical, and physical stressors (Anderson *et al.* 1997; Jin *et al.* 2004; Kay *et al.* 2005; Lleo *et al.* 2005; Evanson and Ambrose 2006).

Enterococcus mundtii might be surviving in the environment because it can remain metabolically stable under a variety of physical stressors. In contrast, lower levels of the feces-associated *E. faecalis* in environmental samples, could be due to the bacteria altering its metabolic state. Since *Enterococcus* is a non-sporulating microorganism, persistence in adverse environments can require a low metabolic activity phase where the cells become viable but non-culturable (VBNC). This allows certain species, such as *E. faecalis*, to persist in the environment but be unrecoverable using normal culturing methods, such as those employed in this study of Oso Creek (Lleo *et al.* 1998, 1999). Heim *et al.* (2002) have suggested that this VBNC state could be a preferred survival strategy of *E. faecalis* in the environment. Thus, this raises the question and potential limitation of what is cultureable in phenotypic studies, and what is actually in Oso Creeks' water and sediments?

Differences among the enterococcal species within the water and sediments of Oso Creek were observed. The most prevalent enterococci from both water and sediments was *E. mundtii*, with *E. faecalis* being the second most prevalent isolate from water and *E. faecium* from sediment (Table 14). During wet events, there was a clear shift in the number and type

of enterococcal species, as *E. faecalis* was isolated more frequently than *E. mundtii* from creek water and sediments (Table 14). The difference in species associated with water and sediments was also found by Evanson and Ambrose (2006) who discovered that sediment associated fecal indicator bacteria populations can be distinct from those found in water samples. One possible explanation for this observation is that enterococcal populations more adapted to surviving in sediments or plant life associated with sediments, may be actually be colonizing these sites and forming environmental reservoirs (Whitman *et al.* 2003). Environmental conditions in tidally influenced sediments support elevated levels of enteric bacteria, thus these waters may serve as sinks for *Enterococcus* which can create an endemic pollution source during tidal and high erosional flow conditions (Desmarais *et al.* 2002; Evanson and Ambrose 2006).

As previously stated, *E. faecalis* is strongly associated with feces and in this study, increased in both water and sediments during wet events, suggesting that fecal matter is loading into the creek during wet events, a phenomenon reported by Crowther *et al.* who demonstrated that fecal indicator bacterial loading can occur during periods of increased moisture (Crowther *et al.* 2001). Bacterial loading has also been shown in previous modeling studies for the Oso watershed (Heilman 2000; Crysup 2002; Campbell 2007). However, persistence of these fecal indicator bacteria once introduced into the environment, along with concurrent persistence of

pathogenic microorganisms, has been less studied and is a key area for future research.

Although a host of taxonomic studies on enterococci exist, the species is still relatively difficult to distinguish phenotypically from other lactic acid bacteria (Fisher and Phillips 2009). Speciation using the MicroLog™ Microbial Identification System detected numerous bacteria besides *Enterococcus* from mE and mEI plates. Frequently, species from the gram-positive bacterial genera *Pediococcus, Lactococcus, Vagococcus, and Streptococcus* were identified. The parallel identification of these genera could be attributed to common pheonotypic traits that are shared between members of the same order *Lactobacillales* (Godfree *et al*. 1997; Fisher and Phillips 2009; Carrero-Colón *et al*. 2011). These findings suggest that although the MicroLog™ Microbial Identification System has been shown to be more accurate than other phenotypic identification systems for enterococci speciation (Moore *et al.* 2006) there is a need for genotypic identification to augment phenotypic methods currently in place.

Differences arise, as seen in species comparisons as well as current statistical modeling, between enterococci isolated in this project and those from a previous study by Stewart (Stewart 2005). However, it must be noted that Stewart utilized different sources of human fecal matter such as portable toilets and human volunteer samples. Stewart also sampled from slaughterhouse cattle, which are under severe stress, a state that alters the physiology of the intestines, likely leading to changes in the gut microbiome (*Enterococcus* included) (Collins 2001; Velin *et al.* 2011). Since Stewart's

human and cow sources differed in type, from this study's human (wastewater isolates) and range cattle, the only truly comparable isolates were dog, as dog isolates in this study were taken at similar sampling sites and locations as those in Stewart's study.

Temporal and geographical stability are unfortunately two of the documented limitations to phenotypic methods, especially ARP and CSU profiling (Harwood 2007; Mott and Smith 2011). Antibiotic resistance traits can change depending on the level of resistance in the environment and within the population that uses them (humans, domestic animals, *etc*.). Plasmid-encoded resistances to antibiotics can result in mixing or altering the antibiotic resistance and susceptibilities within host populations and within environmental sources (Harwood 2007). Further confounding can also result from maintenance of these resistance mechanisms pressuring the organism to ultimately dismiss resistance mechanisms in favorable conditions (*i.e.* no external antibiotic pressure) (Salyers *et al.* 1997; Heinemann *et al.* 2000). Compositions of populations in individual hosts, host populations, and sampling locations can also change over a period as short as weeks (Mott and Smith 2011). These changes can be not only to the species in question, but also to populations of competing or parasitic microorganisms, further altering microbial populations (*i.e. Enterococcus*).

Phenotypic profiles, like ARPs, have been previously demonstrated to be stable for up to a year (Wiggins *et al.* 2003). However, numerous factors can influence temporal variability including transient species, mutations in

host populations, development and urbanization of an area, horizontal gene transfer, and rapid large physical changes that can be brought about by events like flooding or natural disaster. Geographic stability largely influences diversity within species and has been shown to vary between fecal indicators. Generally, it is recommended to use regional libraries, such as state libraries (Mott and Smith 2010).

In order to test the geographical and temporal stability of the Oso Creek library, two sets of isolates from sources outside the watershed were substituted as unknowns (cow and human), obtained by Stewart six to seven years (2003) prior to the animal fecal sampling of this project (Stewart 2005). Since rural areas with limited urbanization surround Oso Creek, it was postulated that there would be limited drifting of phenotypic profiles, as suggested by a previous study (Smith 2009). Results (shown in Tables 25 and 26) demonstrated that geographic and temporal variation had a strong negative effect on classification of unknowns. Stewart's human isolates were classified as seagull and dog, while his cow isolates were classified as dog and seagull in seven-way LDA and RF-stratified models.

Misclassification of Stewart's human and cow isolates as dog and seagull could potentially be caused by the fact that Stewart's dog and seagull isolates were included in the overall Oso Creek library. Thus the cow and human test cases matched best with those collected around the same time from the same study. Justification for including the dog and seagull isolates stemmed from the increased RCC and ARCC values in all classification models, and dog samplings

for both Stewart's study and this Oso Creek study were collected in similar locations throughout the watershed. Stewart's dog and seagull isolates also increased the overall sample size of the Oso Creek library and sample size is an important factor in the validity of phenotypic MST libraries (Johnson *et al.* 2004; Mott and Smith 2011). Furthermore, Stewart's dog isolates classified reasonably well with the current study's dog isolates. Stewart's seagull enterococci did not classify as well with the current study's "other avian" enterococci profiles, and were separated from "other-avian" in seven-way classification schemes.

These temporal and geographic limitations can plausibly explain the poor validation results on the Oso Creek animal isolate library (Tables 25 and 26), although as described previously, neither human nor cow samples were true equivalents, as the types of sources were different and did not have a comparable group of isolates in the library. Taking the isolates from the previous dog samples out of the library and establishing a library to compare these to the isolates from dog samples collected in this study would have provided a more valid assessment. Alternatively, samples in this study could have been collected from similar sources within the watershed and used for validation. These validation tests identify two limitations of phenotypic MST methods and highlight the need for continued updating of MST libraries in contained geographic regions. In order to minimize the possible effects of temporal and geographic haphazards, the majority of the library isolates used for the current study were collected during the same period as the creek isolates and from within the same geographic area.

Sampling and isolation of enterococci from animal sources presents another potential limitation of using phenotypic MST. It is still unclear how many isolates should be isolated per fecal sample, as the possibility exists for clones of isolates to occur in the same sample. This can cause bias when running certain types of statistical analysis, as identical profiles from clones can limit RCCs and overestimate the ability to correctly classify sources of contamination (Johnson *et al.* 2004; Mott and Smith 2011). In order to remove identical isolates from libraries, researchers have used genetic methods, such as ribotyping, to identify clones and remove them from analysis; however, this additional method is costly, time-consuming, and requires significant personnel training to run it (Harwood 2007; Mott and Smith 2011). Ribotyping is also more valid for molecular or nearest neighbor analyses, whereas the LDA clusters are based on similarities and thus it is important to use profiles in the proportions in which they are represented in the animal sources. This study attempted to look at what was "typically" found in the creek. Therefore, a representative sampling of what was in animal feces, clones or no-clones, was used to develop the library.

This study combined CUPs and ARPs to form a composite library, and obtained high ARCCs and RCCs, supporting the use of a MST toolbox approach. Previous *E. coli* MST studies in the region have used both single and combined method libraries of ARPs or CUPs+ARPs (Rifai *et al.* 2005; Wilson 2005; Smith 2009; Smith *et al.* 2010). A study in Houston, Texas included *E. coli* source tracking data using both CUPs and ARPs, and

reported increased ARCCs using a composite database (Rifai *et al.* 2005). A study in Brazoria County, Texas on the Cow Trap and Cedar saltwater lakes, utilized only ARP data and did not include CUP data on the identified *E.coli* isolates (Smith 2009; Smith *et al.* 2010). In this Oso Creek study, incorporating CUPs provided higher discriminatory power with LDA. Interestingly though, RF discrimination decreased as the number of discriminants increased from 21 antibiotics to 116 variables (95 carbon sources in addition to the 21 antibiotics) (data not shown).

The final library was developed using ARCC rates generated from several designed models. Rates of correct classification (RCCs) are averaged from all source categories and comprise the ARCC. However, ARCCs are not a complete measure of the predictiveness of an MST library. As models become more complex, as demonstrated by this and other studies, ARCCs decrease due to the number of categories being compared (Table 27) (Hagedorn *et al*. 2003; Smith *et al*. 2010). Setting priors and strata is one way to increase individual RCCs at the cost of decreasing overall model ARCCs. This serves to boost specific animal groups (human, dog, *etc*.) but setting high priors and strata for specific groups has a negative effect on all other group RCCs as well as overall ARCC for the model. Defined priors and stratification are not explicitly available on SPSS®, but are available on R. Based on current literature regarding LDA, RF, and MST, equal priors for LDA and stratification for RF were used in this study (US EPA 2005; Smith *et al.* 2010).

As evidenced with two-way modeling, the Oso Creek Library was disproportionate when classifying human and non-human sources, with only 111 human isolate profiles versus 974 non-human isolate profiles. Though LDA created a highly discriminant non-overfitting two-way model, RF two-way models between human and non-human source category was less discriminatory, even with stratified sampling. Robinson *et al*. have suggested using the statistical technique *k*-nearest neighbor in cases where disproportionate libraries pose a challenge to traditional statistical methods (Robinson *et al*. 2007). As model complexity grew, RCCs and ARCCs began to decrease; however, overall RCCs and ARCCs in this study compared favorably with those in previous studies using LDA to analyze CSU or ARP data (Hagedorn *et al.* 1999, 2003; Harwood *et al.* 2000; Moussa and Massengale 2008; Smith 2009; Smith *et al*. 2010; Wiggins *et al.* 2003). Other methods have been utilized to maintain levels of discrimination, even with increasing model complexity. Random Forests has not been used widely in MST studies but appears to be a good alternative to discriminate variables and obtain high RCC and ARCC rates. The first published use of Random Forests (in an *E. coli* MST study) showed results comparable to LDA or even better (Smith *et al*. 2010). LDA does have specific benefits; for example it is available on most statistical platforms. Random Forests is currently only available through the open-source command line driven R statistical package, or through special "stand-alone" software. This program requires experience in basic coding language and logic, which could impede initial users but

should not be discouraged due to the powerful statistical packages and open access of the program.

Average rates of correct classification can also be influenced by sample sizes and the representative diversity of the isolates. For these reasons, a high ARCC does not always reflect a representative library (Harwood *et al*. 2003; Wiggins *et al*. 2003; Moussa and Massengale 2008). To avoid this potential problem, other methods of statistically analyzing MST libraries have surfaced. Recently, Smith *et al*. demonstrated that RF could randomly take samples from an MST library, thus removing the element of a potentially overfitting model, and generate higher ARCCs than previously established statistical methods for analyzing MST libraries (Smith *et al*. 2010). However, Smith *et al*. utilized only one type of phenotypic profile, antibiotic resistance profiling (Smith *et al.* 2010). This current study utilized both ARPs and CUPs, which allowed source categories to be grouped together based on their carbon source utilization as well as their antibiotic resistance or susceptibility traits. Use of this more complex database resulted in lower ARCCs and RCCs using RF than LDA, suggesting that the more variables used (such as carbon sources or antibiotics), the less accurate RF becomes. Although RF provided lower RCCs and ARCCs as opposed to LDA, it is still a recommended statistical tool because of resistance to overfitting, which occurred on four of the five created models using LDA (Table 22).

The highest difference between the training and test set for RF-stratified was 4.6%, which was interestingly on the two-way classification model (Table 24). The two-way model often had much lower RCC and ARCC results in comparison to the two-way LDA classification model. However, the RCC and ARCC rates stabilized as the model increased in complexity with the additional break up of animal groups, which has a detrimental effect for LDA (Hagedorn *et al*. 2003; Moussa and Massengale 2008). On this particular CUP and ARP dataset, LDA performed well on simpler models but overfit as animal groups were differentiated in higher models, yet RF was more stable as the models increased in complexity. To note, in classification of unknown source isolates, RF initially started off with almost 40% human classification in two-way models before tapering off to 3.3% in the final seven-way classification model and is a source of concern. Conversely, LDA was able to maintain fairly stable classifications through all models (Table 33). If the two-way model from LDA and the seven-way model for RF are compared, the proportions of human categorized isolates are similar, providing support that human source is not significant in the creek. Therefore, although RF did not overtly outperform LDA as in the only previous study using RF (Smith *et al*. 2010), it was still useful, adding confidence to the results obtained through LDA.

Random Forests was especially useful in situations where cross-validation differences and 80%/20% training and test cases exceeded recommended guidelines for LDA models. This suggests that, LDA and RF can be utilized in tandem to support each other in a “toolbox” effect, similar to utilizing multiple

laboratory methods to compliment phenotypic data as accomplished by other researchers (Casarez *et al.* 2007; Moussa and Massengale 2008), as recommended by Smith (2009), that a combination of both LDA and RF statistical methods be used to increase confidence in results.

Although LDA and RF analysis did show similarities in terms of model creation (Table 27), overall, RF provided lower RCCs and ARCCs for all individual animal groups and classification models. However, as indicated by Tables 22 – 24, LDA overfit four of the five models, in addition to generating differences as much as 11.8% in training and test sets (Table 23). In comparison, RF was resistant to overfitting models and the maximum difference was 4.6% in training and test sets. These validation tests demonstrate the stability of RF over LDA and again suggest that in situations where LDA breaches recommended guidelines (US EPA 2005), RF could be used as a suitable or possibly better alternative as demonstrated in previous MST studies utilizing RF (Smith 2009; Smith *et al.* 2010).

Previous studies utilizing RF have demonstrated that it outperforms LDA in areas of mass spectrometry as well as MST (Wu *et al.* 2003; Smith 2009; Smith *et al*. 2010). Smith postulated that as variables increased, RF would outperform LDA, basing her postulate on previous comparisons conducted between LDA and RF by several researchers which found that the more variables run using RF, the more it outperforms LDA (Wu *et al.* 2003; Guo *et al.* 2004; Lee *et al.* 2005; Pang *et al.* 2006). Smith's MST study utilized 20 ARPs and found similar results that RF outperformed LDA. Smith further postulated

that utilizing CSU data should increase RF performance based on the notion that RF is more robust to outliers than other classification methods (Smith 2009; Guo *et al.* 2004). However, the results of this MST study on Oso Creek do not support Smith's postulate, as with increased variables (116) from enterococcal CUPs and ARPs, LDA outperformed RF.

After testing LDA and RF classification models, unknown isolates from Oso Creek water and sediments were compared with the library in order to classify them into animal categories. Overall, this study identified "other avian" (inland birds other than seagull) and non-avian mammalian wildlife as the primary contributors of contamination in the upper sections of Oso Creek (Table 33), both the main portions of the creek and the secondary tributary, West Oso Creek. Minimal contributions from dog and human were detected, though increased levels of livestock (cow and horse) were found under certain conditions and constituted ~20-30% of contamination depending on classification models. Both LDA and RF models agreed overall on the main sources of contamination and classifications, however, Random Forests did differ slightly from LDA in classifying birds, indicating that "other avian" accounted for ~45% of all contamination within the creek, with a remaining ~28% as non-avian wildlife. In this case, RF should be utilized as the discriminatory model because the seven-way LDA model was found to overfit the data and did not adhere to US EPA recommended guidelines.

Interestingly, during wet and dry sampling for West Oso Creek, which contains farm land and some cow and horse pasture, very high levels of

"other avian" contamination was shown during dry events by RF. A seven-way LDA classified almost 35% of the contamination during dry events as cow and horse, which was expected. Nevertheless, this seven-way model overfit the data as indicated in Table 22 in this instance by 10.9%. Thus, in this instance, it would be beneficial to rely on another statistical method that had lower variability and did no overfit. Some scrutiny should be given to the RF analysis that had lower classification values for cow, as it was anticipated that West Oso Creek might contain a noticeably larger proportion of isolates classifying as livestock (specifically cow) as compared to the main portion of Oso Creek and that wet events are known to result in fecal indicator bacterial loading in coastal waters (Crowther *et al.* 2001).

Additionally, seagulls were not documented nor observed during any of the field surveys or collections within the upstream portion of the Oso watershed. The low rates of classification for seagull in seven-way classification models reflect these field observations. A recommendation for future studies would be to use an animal species found outside the watershed, similar to the use of seagulls in this study, in order to establish a "negative control" for library-dependent MST studies.

In this study, emphasis was placed on obtaining the highest possible RCCs for human classification in all models. Since Oso Creek originates from an effluent based drainage source (TCEQ 2005), misclassification of animal isolates as human would produce false-positive misclassifications. These types of misclassification errors are ultimately the most costly, as a

result suggesting predominant contamination from human sources would likely enact unnecessary and expensive remediation efforts directed toward improving wastewater treatment. Human source (effluent and influent wastewater) was found to be only a very minor contributor to the fecal contamination in the upper creek. These findings are additionally supported by previous investigative efforts for outflow monitoring of the RWWTF that found only rare exceedances of acceptable levels of enterococci (Hay and Mott 2005; Campbell 2007).

Avian and non-avian wildlife have been shown in numerous MST studies as strong contributors of fecal pollution in waterways (Whitlock *et al*. 2002; Choi *et al*. 2003; Graves *et al*. 2007; Somarelli *et al*. 2007; Vogel *et al*. 2007; Smith 2009; Smith *et al*. 2010). Though traditionally, human fecal matter has been considered a larger threat to human health compared with animal waste, because of human-specific pathogens, this view has altered with greater awareness of zoonotic pathogens (Anderson *et al*. 1997; Leclerc *et al.* 2002; Harwood 2007; Meschke and Boyle 2007; Stewart *et al*. 2007; US EPA 2009b). Birds and wildlife are both known vectors of the pathogens *Cryptosporidium parvum, Giardia* spp.*, Campylobacter* spp.*, Leptospira* spp.*, Salmonella* spp. and *E coli* O157:H7 (Meschke and Boyle 2007; US EPA 2009b). Many of these pathogens have been implicated in numerous waterborne outbreaks across the U.S., typically manifesting as mild to severe gastrointestinal illness in humans. However, these disease-causing microorganisms can still be lethal to immunocompromised, young, or elderly individuals.

CONCLUSIONS

This study was the first to analyze coupled enterococcal phenotypic CUPs and ARPs using the statistical method Random Forests, in addition to linear discriminant analysis. Use of multiple lab and statistical techniques in an MST toolbox approach, resulted in successful identification of sources of fecal contamination in the upper section of Oso Creek, Nueces County, Texas. In addition to a library-dependent approach, speciation was shown to be a useful means in understanding the dynamics of animal and environmental sources of enterococci within the watershed.

Further characterization of environmental enterococci associated with plants as well as within water, sediments, and soils is essential to understanding the habitat and survivability of this fecal indicator. Effects of wastewater processing on enterococcal species, along with greater knowledge of metabolic states of different species and persistence of these and pathogenic microorganisms in the environment, are key questions that must be answered in future studies. Though phenotypic libraries have proven successful, care should be taken when constructing libraries in terms of sample size, geographic distribution, and temporal sampling. Updated regional libraries are highly recommended for future MST studies using phenotypic data.

Sources of enterococci within the upper sections of Oso Creek appear to be largely inland bird (non-seagull) and non-avian wildlife, with wildlife sources of enterococci increasing during runoff driven wet events. In

comparison, smaller amounts of livestock contamination and a lack of human fecal contamination were also observed. These sources of contamination suggest remediation strategies for Oso Creek are limited. Fencing, controlled hunting, and trapping could be used to control wild animal populations; however, for Oso Creek this is unlikely to be a realistic approach. As the creek acts as a main source of freshwater for the upstream portion of the Oso watershed and animals will be drawn to it regardless of control measures.

Multiple laboratory and statistical methods were successfully employed and are recommended for future studies. Carbon source utilization was shown to be an effective phenotypic approach for discriminating sources of enterococci within freshwater and sediments. Statistical analysis via Random Forests and linear discriminant analysis proved to be appropriate tools for use with MST phenotypic data. Coupling phenotypic and genotypic MST methods in a "toolbox approach" is a future recommendation. This approach will refine and better develope the science of microbial source tracking, in order to critically examine the sources of fecal pollution within contaminated waterways for the years to come.

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Appendix A
Field Source Information Known Animal *Enterococcus* Isolates

Appendix A Table 1: Latitude and longitude of animal sampling locations

Location	Latitude (N)	Longitude (W)	Animal Type
Agrilife Research Facility	27.7794027	-97.5732110	Bird
Rodeo Run Arena	27.7278083	-97.5007250	Horse
Pee Wee's Animal Shelter	27.7225361	-97.4622610	Dog, Horse
TCEQ Station 18500 | Memorial Gardens Cemetary	27.7300500	-97.5164600	Wildlife, Bird
Hwy 44 & Violet Rd Intersection	27.7840500	-97.5855830	Wildlife
Private Residence (3727 Country Rd 61)	27.7700270	-97.5969720	Cow, Horse
Private Residence (4104 FM Rd 1694)	27.7980500	-97.6183610	Horse
Robstown Waste Water Treatment Facility	27.8002700	-97.6503800	Human
Corpus Christi International Airport	27.7767700	-97.4950830	Wildlife, Bird
Hwy 22 & Hwy 2444 Intersection	27.6795500	-97.4546100	Wildlife
TCEQ Station 18499 | Train Tracks Bridge	27.7840700	-97.5927380	Wildlife
TCEQ Station S2 | Yorktown Rd & Sun Valley Dr Bridge	27.6842050	-97.4226694	Bird
Roadside 7673 Weber Rd	27.6885900	-97.4325200	Dog
Nueces County Animal Control Kennels	27.7969200	-97.6536830	Dog
TCEQ Station 18501 | Fields	27.7095410	-97.5541980	Cow
Roadside 2642 FM Rd 763	27.6958300	-97.5016660	Bird
Nueces Veterinary Hospital (11027 Leopard St) **	27.8416880	-97.5836720	Dog
Private Residence (2065 Co Rd 20A)	27.6536880	-97.5361840	Dog, Horse, Sheep, Bird
Roadside 8600-8698 S Staples St	27.6530410	-97.4099970	Wildlife
Gulf Coast Animal Shelter (3118 Cabaniss Rd)	27.7039780	-97.4300630	Dog
Private Residence (S Violet Rd)	27.7682800	-97.5639500	Cow, Bird, Dog, Wildlife

**Located slightly outside of the Oso watershed.

Appendix B
Field Data Forms

A.) Field Data Form

Environmental Microbiology Laboratory
Fecal Sample Collection
Field Data Sheet

Sample ID Number: _____

Date: _____

Time Collected: _____

Animal Type: _____

Gender (if known): _____

Location Description: _____

Lat/Long: _____

Medication: _____

Collection method: _____

Swab/Container Lot#: _____

Air temperature: _____

Rainfall last 24 hr: _____

Photograph: _____

Comments:

Collector Name: _____

Collector Initials: _____

B.) Infectious Materials Security Plan

Environmental Microbiology Laboratory Infectious Materials Security Plan

For transport of UN 2814 Infectious Substances Affecting Humans

As regulated by federal law 42 CFR part 73 (CDC), Texas A&M University – Corpus Christi Environmental Health and Safety, and per Texas A&M University – Corpus Christi Environmental Microbiology Laboratory (EML) TSSWCB-Oso Creek 07-13 Project QAPP, a security plan is implemented to transfer category UN 2814 "Infectious Substances Affecting Humans." Samples collected from human waste contain unknown pathogens that can adversely affect human health and are therefore classified UN 2814.

All field personnel will adhere to the following guidelines regarding packaging of infectious substances:

- Primary receptacle containing unknown infectious substances will be placed within a secondary watertight packaging containing an absorbent packaging material
- Secondary watertight packaging will have a label indicating list of contents
- Secondary watertight packaging will be placed in rigid outer packaging (ice chest) containing preservation materials if necessary (ice, dry ice, etc.)
- Outer packaging will contain infectious substance label

All field personnel will implement the following safety precautions when transporting infectious substances across city, county, and state roadways:

- Visible hazardous materials UN 2814 sign displayed on vehicle
- Possible security risks include: Theft, Vehicle Malfunction, Detention and Arrest by authorities, and Vehicle Accident etc.
    - To prevent security risks the following measures will be taken by all field personnel:
        - Proper handling technique and personal protection will be worn
        - Vehicles containing infectious substances will be monitored whenever possible
        - Vehicles containing infectious substances will be locked and keys given to field supervisor for safe keeping
        - Only field personnel appointed by EML will transport or come into contact with packages containing infectious substances
        - Proper attention will be given to safe driving and monitoring of safe lawful speeds and road conditions
        - Vehicles will be inspected prior to utilization to check for damages or abnormalities

- In the event that security risks occur the following measures will be taken:
  - Cones will be utilized if vehicle is stopped along roadside
  - If unsafe driving conditions arise, field personnel are to contact EML leadership and pull off the road till conditions become ideal
    - If necessary local or university authorities will be called to create a safe route back to campus
  - If vehicle is stopped by city, county, state, or federal authorities, declaration will be made of transporting human waste and infectious substances, as well as the reasons for collection and transport
  - In the event of an accident or theft, normal protocol (contacting TAMU-CC police, local police, and EML leadership) will be followed with the addition of contacting TAMU-CC EH&S

These procedures will remain in effect till the finalization of the project or at the discretion of EML leadership. All revisions and amendments will be made to this document at the appointed time.

_____
Field Supervisor

_____
EML Technical Director

_____
EML Director

C.) Chain of Custody Form

TEXAS A&M UNIVERSITY CORPUS CHRISTI

Project Leader: Dr. Joanna Mott
Texas A&M University-Corpus Christi

Chain of Custody Form

Environmental Microbiology Lab

Department of Life Sciences
6300 Ocean Drive
Corpus Christi, Texas 78412

Quality Assurance Officer:	Field Supervisor:	Phone:	Fax:	Project Name/Location:	Project No.
LaDonna Henson	Sergio Rodriguez	361-825-3262	361-825-3719	TSSWCB-Oso	07-13

Samplers (sign):

Lab Analysis Request

Sampling Identification No:	Lab Only	Total Containers	Media Code	Matrix: Liquid	Sediments	Fecal Sample	Preserved (w/ice): $Na_2S_2O_3$ Added	Mark "X" on lid	Ice Ref (Only)	Sampling: Date	Time	Total Coliforms SM20 9	Fecal Coliforms SM20 9222-D	*Escherichia coli* EPA1103.1	Enterococci EPA 1600	Clostridium perfringens	mHPC SM20 9215-A	Epifluorescence SM20 9216-B	IDEXX® Enterolert™	Enterococcus Fecal Swab Isolation	Remarks
OST-		1																			
		1																			
		1																			
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		1																			
		1																			
		1																			

Relinquished by: (Print) / (Sign) — Date: — Time:

Received by: (Print) / (Sign) — Date: — Time:

Thermometer ID: Correction Factor: Temperature Blank(s) with Correction Factors Applied:

Temperature Blank Measurement(s):

Appendix C
Carbon Source Utilization and Antibiotic Resistance Profiling Standard Operating Procedures

TAMU-CC BIOLOG™ Procedure for *Enterococcus sp.* samples

Note: These procedures were compiled by TAMU-CC personnel.

Day before running samples:

1. Always use BugB medium and label plates clearly. If transferring from another plate, pull sample from an isolated colony. If transferring from another plate, pull sample from an isolated colony. If transferring from TSA slants, the culture should be pure. Place bacteria in the center of plate and do a triple lawn to evenly distribute cells.
2. Incubate BugB plates for 16-24h at 35°C.
3. Autoclave swabs and pipette tips.
4. Prepare inoculating fluid (20mL/tube, autoclave on a liquid cycle for 30 minutes)

Day of procedure:

1. Remove inoculating fluid tubes and MicroPlates from refrigerator and allow them to reach room temperature.
2. Remove BugB plates in sets of 20 or 40. Label MicroPlates accordingly and add a duplicate MicroPlate every 20 samples
3. Turn on turbidometer and allow it to warm up for 10-15 minutes.
4. Calibrate turbidometer. Manually mix (tilt back and forth) a tube of inoculating fluid and place in the turbidometer. Set transmittance to 100%. Remove IF tube. Manually mix turbidity standard and place in the turbidometer. It should read 20% for the GP-Coc (Gram positive coccus) standard. Get as close to the targeted amount as possible, making sure that the value is within at least ± 5.
5. Wipe a tube of inoculating fluid carefully with a kimwipe and place in the turbidometer. The reading should be right at transmittance at 100%. If not, set to 100% and recalibrate with standard.
6. Open a new tube of thioglycolate (an anticapsulating agent) (caution: this substance is highly toxic). To do this, hold reagent dropper upright and point tip away from yourself. Squeeze middle gently once with thumb and forefinger to crush ampule inside the dropper.
7. Dispense 3 drops of thioglycolate into inoculating fluid. Do not use more than 3 drops per 18-20mL of fluid.
8. Moisten a sterile swab with inoculating fluid. Roll swab over the colonies rather than sliding across them. Be sure not to pick up any agar. Twirl the swab against the inside surface of the tube (above the fluid line) to gently break up clumps. Place swab in fluid. Swirl swab in fluid with a turbulent vertical motion to the bottom of the tube to create a uniform suspension, avoiding the sides of the tube. Cap tube tightly and invert tube 5 times to evenly distribute the bacteria. Do this carefully. Inoculum must be homogenous and free of clumps. If bubbles appear, wait for them to settle, or the reading will be inaccurate.
9. Read turbidity. It should be within ± 2 of the turbidity reading of your standard. If it is too high, add more bacteria using the procedure above. If it is too low, add more inoculating fluid with a sterile disposable pipette. Invert tube 5 times and read again. Repeat as necessary.
10. Once turbidity is in range, pour inocula into a reagent reservoir. Add tips to micropipette. It should be set to 1250µL (should be program 1).
11. Place pipette tips into reservoir and press “Fill.” Inocula should be drawn into tips.
12. Align tips with first row of MicroPlate and press gray button on the handle. Repeat this procedure for other rows. When you run out of fluid, press “Purge” button. Place micropipette tips over reservoir and push gray button to release fluid.
13. Repeat procedure until all rows are filled.
14. If any MicroPlate wells are not full, fluid can be added using a sterile disposable pipette. Any overflow should be removed with a sterile swab.
15. Place lid on MicroPlate and incubate at 35°C for 16-24h. Record log in information on the incubator log sheets.

Reading plates:

1. Open the Biolog 420 program and under INPUT screen select
    a. reader
    b. MicroStation2
    c. Comport 1
2. For each plate, fill in the following data
    a. Plate Info (pull-down menus—info is required for plate reading)
        i. Plate type (GP)
        ii. Strain type
        iii. Incubation time
    b. Plate Info (defined by user—optional)

i. Sample number, Strain name, Strain number, Other

3. Plate reader must be on. After turning on, the plate reader will self-calibrate. After the self-calibration is over the reader will beep and the screen on the plate reader will say ready.
4. After self-calibration is complete, click the initialization button once. Initialization should be complete in a minute or two. The reader ready should change to yes on the computer monitor.
5. After initialization is complete, remove MicroPlate lid and insert into reader snugly.
6. Click read.
7. After reading, a circle with a horizontal line through it means the well was negative and a circle with a plus sign means the well was positive.
8. The id is based on a progressive database which is based on the number of reactions in the plate; the specific pattern is what the mismatches are based on and the v. current MicroPlate gives an idea where mismatches come from

TAMU-CC Antibiotic Resistance Analysis Protocol

Follow procedures of Clinical and Laboratory Standards Institute (CLSI)

CLSI (2006) Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Ninth Edition. CLSI document M2-A9.

CLSI (2008) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard-Third Edition. CLSI document M31-A3.

CLSI (2006) Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. CLSI document M100-S16

Quality Assurance/Quality Control Protocol

EQUIPMENT AND SUPPLIES
For data to be reliable, standard QA/QC must be followed in the lab. All equipment (*i.e.* incubators, biohood, refrigerators) and supplies used must be maintained according to QA/QC standards.

Antibiotic disks must be kept in the freezer at -14°C or below until needed (M2-A9, p9). Disks may be stored in refrigerator at 8°C or below. However, drugs from B-lactam class (AMC, AM) should be stored in fridge no longer than a week. Other labile antibiotics (IPM) should also remain frozen. A small working supply placed into the disk dispensers can be kept in the refrigerator as long as they are stored in a tightly sealed desiccated container. An individual antibiotic tube of each antibiotic may be kept in the refrigerator in case the dispenser needs to be changed and this will allow for a quick warming time. These antibiotics may only stay in the refrigerator for up to one week. If you must open a new box of antibiotic cartridges, look for the box that has the nearest expiration date. When a box is finished, please remember to update the Drug Log on BIOMIC. Always make sure to enter media and antibiotics in BIOMIC as they arrive. Log into BIOMIC, click on “logs”, and update the system. If a box of disks expires, let the project manager know. Do not throw expired disks away; notify lab manager.
Disk dispensers are kept in the refrigerator and must be taken out and allowed to reach room temperature before opening. The extra antibiotics may also be removed from the refrigerator in case you need to change the dispenser while plating. A metal desiccator should always be in the dispenser case. This desiccator has blue beads inside, which turn pink when saturated with moisture. If you notice the beads are pink, heat the desiccator at 121^{o}C for 2-3 hours in the grey, dry oven in the main lab (DO NOT USE THE AUTOCLAVE.). When using the dispensers and an “X” on the antibiotic disk in observed, the antibiotics must be changed. The dispensers should be cleaned each time the cartridges are changed. The cleaning protocol and recipes for reagents involved in this process follow below. Stock solutions of Sterile DI water and 3% disinfectant must be maintained and may be kept on the shelf for up to three weeks.

<u>To prepare sterile DI water:</u>
Fill two 1 liter flasks with NANOpure water.
Autoclave on a 15 minute cycle for sterility.

<u>To prepare a 3% disinfectant solution:</u>
Pour 30mL of Lysol disinfectant found into a 1 liter flask

Fill the remaining (970mL)with NANOpure water to make 1 liter.

To prepare an 85% isopropyl solution:
Pour 850mL isopropyl alcohol into a 1L flask.
Add 150mL reagent water to flask.
Note The 85% isopropyl alcohol must also be used to clean the dispensers, but this must be kept in the flammable cabinet and not as a stock solution on the shelf.

To clean the stampers

1. Once antibiotic canisters display an "X", they must be replaced. This "X" represents the last antibiotic in the sleeve of 50 antibiotics. Move switch on tamper to "unlock" position. Pull out and throw out empty canisters into biohazard trash bin.
2. Set out four empty (no media in them) 150 X 15 mm petri dishes. Fill one about halfway with Lysol® disinfectant solution. Fill the second halfway with 85% isopropyl solution. Fill the last 2 halfway with sterile DDI water.
3. Place stamper directly over first plate and push lever down completely. Make sure white dispenser tabs touch liquid. Leave submerged for 30 seconds.
4. Repeat with last 3 plates. Allow dispenser to air dry. The dispenser may be dried on the underneath side where the disks come out with sterile swabs.
5. Refill dispenser with appropriate antibiotic canisters. Once refilled, make sure all of the antibiotic canisters are pushed down and slide the switch into "lock" position.

CONTROLS
Controls are run with each set of samples and anytime the lot number of media, plates, or antibiotic disks is changed. The *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Ninth Edition* (or edition is current to date), indicate the control strains to be used. Check with this publication to see which ones are current for the type of bacterial samples being run. For *Enterococcus sp.* analysis, the minimal QC recommendations from CLSI is *Staphylococcus aureus* (ATCC 25923) (M100-S16, p52). Controls should be maintained on TSA slants in the culture fridge. These slants may be used for up to three months.

MEDIA PREPARATION
Media should be kept in the proper cabinet to avoid moisture. Directions for making media are on the bottles and described below. Media should be put back in its proper place when finished so that it can be located by everyone. Make sure to log the media for antibiotic resistance in the current media log sheets folder. When a new bottle of media is opened it should be noted in the Media Log in BIOMIC. The bottle of media with the closest expiration date should be opened. The date the media is opened needs to be written on the bottle along with the initials of the person who opened it. All media for this project has straightforward directions on the bottle. NANOpure water must be used and the pH must be checked for each flask of media made. Make sure the pH and conductivity of the NANOpure water has been checked before use.

Mueller Hinton I Agar
Mueller Hinton I agar is used for the plates used for the antibiotic analysis. Each isolate will require approximately 140mL (for two plates), plus additional plates for controls.

After autoclaving, set one flask under the biohood and the other flasks onto hot plates on low heat and low stir to keep them from solidifying.

Use 150x15mm petri plates when pouring Mueller Hinton I agar. Do not throw away the bags for these. They will be used for storage once the plates are solidified.

Set each plate flat, and pour media just to the line in 150mm plates under the biohood (~60-70mL per plate). Do not stack the plates after pouring. Set the lid ajar so that the condensation may escape as the media solidifies. Condensation in the plates may dilute the concentration of the bacteria when plating.

Once the plates are solidified, invert the plates into the plastic bags.
Tape the bags shut, label the tape with the date, media type and project name and store media in the refrigerator. These plates are good for up to two weeks.

Place one plate immediately into 35^{o}C incubator and check for sterility after 24 hours.

TSB Broth
TSB broth is used in the sample preparation. Nutrient broth is also acceptable to use.

Each isolate will require 5mL for each tube and 3-4mL for the initial sample preparation. Additional broth will be required to adjust the turbidity of the sample and for preparing controls. Before autoclaving, pipette broth (1 tube for each isolate and control, with extras for emergency) into 16x125mm disposable test tubes (5mL/tube) and cap the tubes. Pour the remaining broth into 125mL flasks (50-75mL/flask). Place foil over the flasks or put screw-cap on (depending on flask in use). Place autoclave tape over the flasks and a long strip over the test tube caps.

Set media into the autoclave on a 15-minute liquid cycle.
Label all media with date, initials, and type of media. Refrigerate in media refrigerator until used (4-8°C). Flasks with screw-caps are good for up to 3 months. Flasks with foil only and disposable tubes are good for up to 2 weeks. Discard media if color change is noted or contamination occurs.

TSA Plates
TSA plates are needed for the preparation of samples. Samples will be streaked prior to inoculation of broth to ensure fresh growth. To maximize usage of media, up to eight streaks can be made per plate if plate is divided into segments.

TSA plates can be stored in fridge up to two weeks.

SAMPLE PREPARATION (Day before procedure)

When preparing samples you must allow them ample time to grow. You can use samples from slants or from cryofreeze. To transfer:

1. UV sterilize the hood for 15 minutes. Turn UV light off and clean working surfaces with Sporocidin.
2. Invert TSA plate and split into 8 even sections using a Sharpie. Label each section with a sample number.
3. Collect supplies. Sterile loops (1uL for cryovials, 10uL can be used for slants) or needles may be used to transfer the cells to the agar plates.
4. If using cells from cryo, remove only a few samples from the freezer at a time to avoid thawing. Continually thawing and refreezing may break cells and decrease viability of the sample.
5. Remember to always use aseptic technique**.** Take the vial from the freezer, open the cap to the vial and collect a small amount of the sample. Streak the cells onto a section of the TSA plate. Use the same procedure if collecting from slants. Place plates back into appropriate refrigerator and vials back into cryofreeze as soon as possible. Controls are usually taken from working cultures on TSA slants. Remove only the slants needed for transfer from the refrigerator. Allow the cultures to come to room temperature before transferring to the TSA plate.
6. Set the samples into the rotator in Incubator 10 or in a rack in tabletop shaking incubator (35°C). Place them in the rotator in numerical order going clockwise from the “Start” sticker. Log info on the incubator log sheet. To maintain a constant incubator temperature, it may be helpful to remove the metal tube holder from the rotator while loading the samples. The tube holder is removed by carefully unscrewing the black knob in the center of the rotator and carefully removing the metal holder.
7. If using the bench top incubator, turn the incubator on (located on the bottom right side of the machine), push the left arrow twice until the screen reads: select program, choose P1, and then push start. Be sure to number beakers in order of the sample numbers they contain. Log info on the sheet provided next to the incubator.

*If time allows, label the TSB tubes with the sample numbers for the next morning.

SAMPLE PREPARATION (Day of procedure)

Samples usually need 2-6 hours of growth in TSB before they can be plated, so transfers must start early.

Sterilize hood with disinfectant and UV it for 15 mins. During this time, set out your TSB tubes.

1. Remove TSA plates the next morning. Sign out on incubator log sheet.
2. If TSB tubes have not been labeled, do this now.
3. Use a sterile inoculating loop or needle and aseptically transfer cells from the TSA plate to the corresponding TSB tube.
4. Incubate TSB tubes at 35°C for 2-6 hours.

PREPARATION

Several materials must be brought to room temperature before you can proceed; set out disk dispensers (with disks), extra antibiotics, sterile transfer pipettes, sterile TSB flasks and Mueller Hinton plates a few hours before you plan on beginning.

1. Divide the antibiotics into 2 groups based on which stamper they will be used in. Bag these sets in Whirl-pac or Ziploc bags and set aside.
2. Autoclave swabs and 13X100mm tubes (cuvettes: 7 per self-seal bag) for 15 minutes at 121°C on a gravity cycle.
3. Cut parafilm into 1-inch square pieces and place into a large, empty weigh boat. You will need at least one square per sample, with extras for duplicates and mistakes.
4. Make sure you have enough cleaning supplies for stamper as outlined in the earlier Quality Assurance/Quality Control section.
5. Each tube in the shaker will have two plates for each drug panel; 1 and 2. Make sure plates are free of excess surface moisture. Place in incubator (35°C) or biohood with lids ajar about 10-30 minutes for moisture to evaporate (M2-A9, p8). Label the side of the bottoms of the plates with sample ID and number (1 or 2). For each 10th sample, label duplicate plates (1 &2) for that sample number. For example, if you have 50 samples, you will have 5 duplicates. You can label duplicates as the same sample number with “DUP” after it. Invert plates and bag them until needed. Label bags in order of sample numbers. Plates are usually bagged with 10 samples and their duplicate. Prepare duplicates of each of the controls (not to be counted as the 10% of samples) to ensure that one of the antibiotics does not fall off of a control, making that control void.
6. Turn on the spectrophotometer and set transmittance to 625nm. Let it warm up for 1 hr. Always ensure the spectrophotometer is level.
7. To calibrate:
    a. Use a 13x100mm tube (cuvette) filled with reagent water as the blank. Wipe outside surface of tube with a Kimwipe and cover with parafilm. Tube must be smudge free when placed into the spec. Put the blank into the spec and put the cap of the spec down. With the Milton Roy Spectronic 20D+, use the right knob to set the transmittance to 100%. Once it reaches 100%, set the mode to absorbency. The spec. should now blink 1.999. With the Thermo Spectronic Genesys 20, simply put the blank in and hit the “0 ABS/100% T” button and it will read “setting blank” until done.
    b. Leave on absorbency mode. Vortex McFarland turbidity standard No. 0.5. Wipe outside surface of tube with a Kimwipe and place into spec. Absorbency should be between 0.08 and 0.10. Blanking with pure water is necessary to ensure the McFarland standard is within guidelines.
    c. Since the actual samples will be done with TSB, it is necessary to blank the spectrophotometer again. Repeat Step 7a with a cuvette tube of uninoculated TSB using a sterile transfer pipette.
8. Place samples, TSB flasks, swabs, transfer pipettes, plates, spectrophotometer tubes (cuvettes), extra test tube racks and parafilm in the biohood. Plug vortex in and set up where convenient.

PLATING SAMPLES

1. In the biohood, use a transfer pipette to transfer small amount of TSB (~3-4 ml) into cuvette. (You want enough liquid in the cuvette for the spectrophotometer to be able to pick up an absorbency reading.) Use a new pipette to transfer 2-3 drops of sample (from inoculated TSB tubes) into cuvette. Place parafilm over top of tube to seal, making sure to only let the side resting against the parafilm paper to rest face down towards the sample. If anything else comes into contact with the side of parafilm resting against the parafilm paper, the sample could become contaminated during vortexing. Vortex and wipe tube with a Kimwipe. Place in

spec and read absorbency. Absorbency should be between 0.08 and 0.10. If too high, carefully remove parafilm and aseptically add TSB from flask with transfer pipette. Parafilm, vortex, wipe tube, and read in spec again. If too low, add cells from sample tubes with transfer pipette. Parafilm, vortex, wipe tube and read again. After a few rounds of this, one will get a feel of the ratio of TSB to drops of sample, which is dependent upon the turbidity of the inoculated TSB samples. Theoretically, most of the samples should be approximately the same turbidity since they were all incubated for the same amount of time. It is best to do the controls first.

2. No more than 15 minutes after proper absorbency is reached place sterile swab in broth (M2-A9, p10). Rotate swab on side of tube to remove excess inoculum. If before swabbing, there is excess condensation on the plate, obtain a sterile swab and dab off excess moisture. Multiple swabs may be necessary if plate is too moist. Inoculate Plate 1 by streaking the swab over the entire agar surface (referred to as "complete lawn.") Repeat two more times rotating plate 60 degrees each time. Set the plate aside, invert and begin to stack them.
3. Repeat Step 2 for Plate 2. It may help to keep Plates 1 and 2 in separate stacks.
4. Allow broth to absorb 3 to 5 minutes (but no longer than 15 minutes) on the MHA plates before dispensing disks (the apparatus is referred to as disk tampers or stampers). To stamp the plates, place the disk stamper over the sample with the lid off and media side up. Make sure all the antibiotic sleeves are in the correct positions and the switch is in the "lock" position. Push the lever (top of apparatus) down carefully and steadily to ensure proper release of the antibiotics. Stamp Plate 1 with the stamper loaded with antibiotic group 1. Stamp Plate 2 with the stamper loaded with antibiotic group 2. Leave the plates right-side up under the hood for least 5-10 minutes so that the disks may set onto the media. In case not all of the disks come out simultaneously, flame sterilize forceps and use these to remove the proper undispensed antibiotic from the tamper and place in correct position on plate. Do not slide the antibiotics across the media surface when manually placing them. This could affect the results of the antibiotic resistance analysis.
5. Carefully remove plates from hood without disturbing the antibiotics. Carefully invert plates and place in 35°C incubator for 16 to 18 hours and log on the sheet provided. It is necessary to use extreme caution when doing this because if one antibiotic out of the whole set (usually 20) falls off, the entire sample is unusable for that day. The entire panel of antibiotics must be performed the same day per sample for accuracy and precision purposes.

READING PLATES WITH BIOMIC

BIOMIC is a computer based plate analyzer. The plate is photographed and zones are measured and interpreted by the computer.

1. Open BIOMIC (it has a biohazard symbol as its icon.)
2. Read the control plates first. Click on "New QC," which stands for Quality Control. Fill out Organism (drop down to proper species and ATCC number of the control organism), Initials (your initials), and Drug Panel (group 1 or 2). Ensure the test date is correct. This program walks one through the process with directions in the left menu panel. Open the drawer and line up the proper antibiotic with the arrow in the drawer. Click "Read plate", make sure all the antibiotics are in the proper place as the computerized zone diameter circles (adjust zones accordingly if need be), and click "View results". If the controls were done properly, all of the fonts should be green and say "OK" under the Quality column. Sometimes it says "N/A" for certain antibiotics for certain control organisms. If the control is within correct specifications, print test (if necessary), and hit "New QC" and repeat step 2 for both panels of all the control organisms. If all control organism QC data is satisfactory, it is not necessary to analyze the duplicates of the control organisms. The purpose of the duplicates of the control organisms is to ensure either the original or the duplicate came out with all 20 antibiotics in the proper place.
3. For the regular samples, click "New Specimen Test".
4. Fill out Specimen #, Technician (you), Supervisor, Specimen Type (*e.g.*, stool), Organism Group (*e.g.*, gram negative enteric), Organism (*e.g.*, *E. coli*), Drug Panel (group 1 or group 2), and any Comments about the appearance of the plate.
5. Open drawer and place the plate on the reading tray making sure that plate is lined up correctly and correct Drug Panel was selected. The computer will prompt you on the proper way to place the plate, but if it is a group 1 antibiotic then the orange arrow on the reading tray must be lined up with AMC 30, if it is a group 2 then it must be lined up with CZ 30.
6. Click "Read Plate".

7. Observe results to ensure that all zones were read properly. Adjust as necessary.
8. Print page if necessary, click "Save" (the program usually saves automatically anyway, but just to be safe) and "Start New Test".
9. If at anytime something is not right, click "Discard test" and start over. Returning to the Main Menu at anytime leads to other menu options as well.
10. Once a sample has been run, it can be accessed under "Current Batch." Double clicking on the sample number allows access to the sample. There are colored tabs at the bottom left of the screen. If a sample ID was typed in wrong, it can be changed under the "Information" tab.
11. It is vital to make sure both plate 1 and plate 2 of each sample are available for analysis. If one is not, then the other should not be read either. Both drug panel sets must be ran and read in the same day to be valid. Once all of the plates have been read for the day, they need to be placed into triple bagged biohazard bags for disposal. No more than approximately 30-40 plates should be placed into a triple-bagged container due to the large volume of MHA that will melt during autoclaving. The bags should be secured shut and have autoclave placed on it with room number, initials, and date written on the tape with Sharpie. All bags should be autoclaved within one week.

MAINTAINING THE DATABASE

It is very important for the database of information to be maintained. It is also important to keep a hard copy (print out) of all data. BIOMIC will automatically backup any data once per day. Once you are done with a batch you need to send the current batch to the past. Do this by scrolling down the current batch, highlighting the last entry, right click, choose select all, right click again, then choose move to past. BIOMIC will automatically save a back up to the hard drive one time each day as you close out of the program. Occasionally, a backup copy (cd or flash drive) must be resaved to keep the raw data updated.

IMPORTING DATA INTO EXCEL

1. Open the BIOMIC program.
2. Click Transfer in the top menu toolbar.
3. In the dropdown menu, click "Data Export".
4. It gives a test date range. It is important to note here that one can only pull by test date range, not by sample type or ID numbers. If a large amount of data is pulled, it may take a long while, and it is recommended to do it by smaller test date ranges. Enter the test date range required.
5. Click all pertainable checkmark boxes next to the fields required. SIR interpretation refers to "Susceptible, Intermediate, or Resistant" zone diameters. Test Date, Specimen Type, Drug Panel Name, and Zone Diameter are almost always recommended.
6. Click Begin Export. This will prompt a Save window to come up. The file is initially saved as a text file (*.txt) and needs to be named appropriately in the correct file. Click save when ready. The Save window will go away now. When it is done, click Okay in the original window.
7. Close out the BIOMIC program.
8. Open Microsoft Excel.
9. Click Data in the menu toolbar.
10. In the dropdown menu, click Get External Data. From the side menu, click Import Text File.
11. An Open File window will come up. Find the text file just saved in the appropriate save location. Double click on file or click Import.
12. The Text Import Wizard window will pop up. It should say Step 1 of 3. Click Delimited, and start window at Row 1. Hit Next. Under Step 2, click the Tab and Comma boxes only. Hit next again. Under Step 3, it should be clicked on General, and one should only have to hit Finish.
13. Then, it can either be opened in the existing worksheet or a new worksheet. Hit Okay.
14. The data should appear in columns; if not, start over. It is important to note that the data becomes imported as all of one drug panel in rows followed by the other drug panel underneath. Scroll down halfway to get to the other drug panel. It is usually desired to line up the drug panels so that all 20 antibiotic zone diameters or SIR interpretations are side-by-side next to the sample number. It takes very careful and meticulous cutting and pasting to make sure that all of the data of a given sample stays together. Any minor mistake will have detrimental effects on the statistical analyses performed.
15. Once the required database is complete, it should manually be checked, zone diameter by zone diameter of every single sample against the printed results, which should be kept in three-inch binders in the lab or the lab coordinators office. Ideally, and at the discretion of the project manager, the numbers should be completely checked by two separate persons to be absolutely sure of accuracy of data.

Appendix D
R Statistical Package Code

```
#Sergio Enrique Rodriguez
#OST-Project & Thesis Results R-Code
#October 8th, 2011
##Models included both ARA and CSU data, featuring 116 discriminants.
##This script runs Linear Discriminant Analysis, Cross-Validation,
## Defined Priors Linear Discriminant Analysis, Random Forests,
## Random Forests-Stratified, and other tools which assisted in classification.
##This code is set up for 118 categories, 2 description categories (isolate name
## and isolate type [1 ... 7]), 95 carbon sources and 21 antibiotics.
##This file was adapted from code created by Professor Blair Sterba-Boatwright, PhD.
##This script utilizes the following libraries: library(MASS), library(randomForest)
##This script utilizes the following scripts created by B. Sterba-Boatwright:
## source("multiDensityPlots.R"), source("crossPlot.R"), source("GAPplot.R"),
## source("kFoldSplit.R").
##All comments are designated by "#" sign.


#############################################################################
#############################################################################
#2-Way Model: Human:Effluent(OST:H-e) vs NonHuman(OST-B,C,D,E,W + BST-B,D)
#############################################################################
#############################################################################

##----------Linear Discriminant Analysis Set-Up
library(MASS)
ost<-read.csv("ost2way.csv")
ost.known<-ost[!is.na(ost$Type),2:118]
ost.known$Type[ost.known$Type==1]<-"Human"
ost.known$Type[ost.known$Type==2]<-"Non-Human"
ost.known[,1]<-as.factor(ost.known[,1])
table(ost.known$Type)

##----------Graphically Surveying Data
source("multiDensityPlots.R")
multi.density.plots(ost.known[-1],ost.known[,1],2,5)
source("crossPlot.R")
cross.plot(ost.known[,-1],ost.known[,1],2,5)
source("GAPplot.R")
source("kFoldSplit.R")
o.split<-k.fold.split(ost.known[,1],4)
GAP.plot(ost.known[o.split==1,-1],ost.known[o.split==1,1])

##----------Linear Discriminant Analysis
ost.lda<-lda(Type~.,data=ost.known)
ost.lda.pred<-predict(ost.lda,dimen=4) #DA-Even Priors
(ost.lda.table<-table(ost.known$Type,ost.lda.pred$class))
(ost.lda.rcc<-diag(ost.lda.table)/apply(ost.lda.table,1,sum))
(overall.lda.rcc<-sum(diag(ost.lda.table))/sum(ost.lda.table))

ost.lda.cv<-lda(Type~.,data=ost.known,CV=T) #DA-Cross Validation
(ost.lda.cv.table<-table(ost.known$Type,ost.lda.cv$class))
(ost.lda.cv.rcc<-diag(ost.lda.cv.table)/apply(ost.lda.cv.table,1,sum))
(overall.lda.cv.rcc<-sum(diag(ost.lda.cv.table))/sum(ost.lda.cv.table))

ost.lda.pred.p<-predict(ost.lda,prior=c(0.58,0.42),dimen=4) #DA-Defined Priors
(ost.lda.table.p<-table(ost.known$Type,ost.lda.pred.p$class))
(ost.lda.rcc.p<-diag(ost.lda.table.p)/apply(ost.lda.table.p,1,sum))
(overall.lda.rcc.p<-sum(diag(ost.lda.table.p))/sum(ost.lda.table.p))

ost.lda.cv<-lda(Type~.,data=ost.known,prior=c(0.58,0.42),CV=T) #DA-Cross Validation w/Defined Priors
(ost.lda.cv.table<-table(ost.known$Type,ost.lda.cv$class))
(ost.lda.cv.rcc<-diag(ost.lda.cv.table)/apply(ost.lda.cv.table,1,sum))
(overall.lda.cv.rcc<-sum(diag(ost.lda.cv.table))/sum(ost.lda.cv.table))

##----------Random Forests
library(randomForest)
ost.rf<-read.csv("ost2way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Non-Human"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

table(ost.known.rf$Type)
ost.k.rf<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T) #RF
ost.k.rf$confusion
(ost.k.rf)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(72,60)) #RF-Stratified
for sample sizes
ost.k.rf.s$confusion
```

```
(ost.k.rf.s)

##----------Check for Dups and Set-up for 80%/20 Validation
x<-read.csv("ost2way.csv")
x[,2]<-as.factor(x[,2])
dim(x)
just.meas<-x[,-c(1,2)]
dist.x<-as.matrix(dist(x),1046,1046)
diag(dist.x)<-1000
min(dist.x)#  no duplicate rows

#Takes 20% of Known Library out and test's it against the remaining 80% for both LDA and RF
#loop 100 times testing LDA and RF w/20% each
source("kFoldSplit.R"); library(MASS); library(randomForest)

lda.rcc<-rf.rcc<-matrix(0,100,2)
for (i in 1:100) {
        splitz<-k.fold.split(x[,2],2)  #  keeps proportions of each class
        train.set<-x[splitz!=1,]
        test.set<-x[splitz==1,]
        lda.model<-lda(Type~.,data=train.set[,-1],prior=c(0.58,0.42))
        lda.test<-predict(lda.model,test.set)
        lda.table<-table(test.set$Type,lda.test$class)
        lda.rcc[i,]<-diag(lda.table)/apply(lda.table,1,sum)
        rf.model<-randomForest(Type~.,data=train.set[,-1],strata=train.set$Type,sampsize=round(0.50*c(72,60)))
        rf.test<-predict(rf.model,test.set)
        rf.table<-table(test.set$Type,rf.test)
        rf.rcc[i,]<-diag(rf.table)/apply(rf.table,1,sum)
        print(i)
}

lo.pct<-function(x) {quantile(x,0.025)}; hi.pct<-function(x) {quantile(x,0.975)}
lda.pct.0.025<-apply(lda.rcc,2,lo.pct); lda.pct.0.975<-apply(lda.rcc,2,hi.pct); lda.mean<-apply(lda.rcc,2,mean)
rf.pct.0.025<-apply(rf.rcc,2,lo.pct); rf.pct.0.975<-apply(rf.rcc,2,hi.pct); rf.mean<-apply(rf.rcc,2,mean)
species<-c("Human","Non-Human")

plot(c(0,1),c(0,3),type="n",ylab=NA,xlab="means and 95% intervals for rcc",sub="red for lda, blue for rf")
for (j in 1:2) {
        lines(c(lda.pct.0.025[j],lda.pct.0.975[j]),rep(j-0.1,2),col="red",lty=2)
        text(lda.pct.0.025[j],j-0.1,"(",col="red");text(lda.pct.0.975[j],j-0.1,")",col="red")
        points(lda.mean[j],j-0.1,pch="*",col="red")
        lines(c(rf.pct.0.025[j],rf.pct.0.975[j]),rep(j+0.1,2),col="blue",lty=2)
        text(rf.pct.0.025[j],j+0.1,"(",col="blue");text(rf.pct.0.975[j],j+0.1,")",col="blue")
        points(rf.mean[j],j+0.1,pch="*",col="blue")
        text(lda.mean[j],j,species[j])
}

(overall.lda.rcc<-sum(diag(lda.table))/sum(lda.table)) #Computer ARCC for LDA
rf.model #Computer ARCC for RF (remember to subtract OOB error rate from 100% to get ARCC)

##----------This generates RF model 100 times and estimates the RCC ranges for each class.
rf.rcc.2<-matrix(0,100,2)
for (i in 1:100) {
        rf.model<-randomForest(Type~.,data=x[,-1],strata=x$Type,sampsize=c(72,60))
        rf.rcc.2[i,]<-1-rf.model$confusion[,3]
        print(i)
}

lo.pct<-function(x) {quantile(x,0.025)}; hi.pct<-function(x) {quantile(x,0.975)}
rf.pct.0.025<-apply(rf.rcc.2,2,lo.pct); rf.pct.0.975<-apply(rf.rcc.2,2,hi.pct); rf.mean<-apply(rf.rcc.2,2,mean)
species<-c("Human","Non-Human")

plot(c(0,1),c(0,3),type="n",ylab=NA,xlab="means +/- sd and 95% intervals for rcc",sub="red for lda, blue for rf")
for (j in 1:2) {
        lines(c(rf.pct.0.025[j],rf.pct.0.975[j]),rep(j,2),col="blue",lty=2)
        points(rf.mean[j],j,pch="*",col="blue")
        text(rf.pct.0.025[j],j,"(",col="blue");text(rf.pct.0.975[j],j,")",col="blue")
        print(paste(species[j],"=",signif(rf.mean[j],3),"+/-",signif(apply(rf.rcc.2,2,sd),3)))
        text(rf.mean[j],j+0.25,paste(species[j],"=",signif(rf.mean[j],3),"+/-",signif(sd(rf.rcc.2[,j]),3)))
}

##----------Using the 2-Way Model for Unknowns using LDA
library(MASS)
ost<-read.csv("ost2way.csv")
ost.known<-ost[!is.na(ost$Type),2:118]
ost.known$Type[ost.known$Type==1]<-"Human"
ost.known$Type[ost.known$Type==2]<-"Non-Human"
ost.known[,1]<-as.factor(ost.known[,1])
table(ost.known$Type)
```

```
ost.u<-read.csv("unknowns.csv")
ost.unknown<-ost.u[!is.na(ost.u$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown<-ost.unknown[!is.na(ost.unknown[,1]),]
ost.unknown$Type[ost.unknown$Type==8]<-"Unknowns"
table(ost.unknown$Type)

ost.lda.hnh<-lda(Type~.,data=ost.known)
ost.plda.hnh<-predict(ost.lda.hnh,ost.unknown)
(ost.lda.table<-table(ost.unknown$Type,ost.plda.hnh$class))
#fp.hnh<-row.error.rate(ost.lda.table,"Non-Human")
#fn.hnh<-row.error.rate(ost.lda.table,"Human")
#acc.hnh<-table.correct.rate(ost.lda.table)

##----------Using the 2-Way Model for Unknowns using RF
library(randomForest)
ost.rf<-read.csv("ost2way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Non-Human"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("unknowns.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T) #RF
ost.u.rf<-predict(ost.k.rf,ost.unknown.rf)
table(ost.u.rf)
#(ost.rf.table<-table(ost.unknown.rf$Type,ost.u.rf$class))
#ost.k.rf$confusion
#(ost.k.rf)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(72,60)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 2-Way Model for Unknowns - Wet Events using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost2way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Non-Human"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-W.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(72,60)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 2-Way Model for Unknowns - Dry Events using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost2way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Non-Human"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-D.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(72,60)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 2-Way Model for Unknowns - Dry Events Stations 499, 500, & 559 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost2way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Non-Human"
```

```
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-D-499-500-559.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(72,60)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 2-Way Model for Unknowns - Dry Events Stations 501 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost2way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Non-Human"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-D-501.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(72,60)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 2-Way Model for Unknowns - Wet Events Stations 499, 500, & 559 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost2way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Non-Human"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-W-499-500-559.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(72,60)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 2-Way Model for Unknowns - Dry Events Stations 198 & 501 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost2way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Non-Human"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-W-198-501.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(72,60)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 2-Way Model for Human Unknowns for External Isolate Validation
library(randomForest)
ost.rf<-read.csv("ost2way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Non-Human"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("bsthuman.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)
```

```
ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(72,60)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 2-Way Model for Bovine Unknowns for External Isolate Validation
library(randomForest)
ost.rf<-read.csv("ost2way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Non-Human"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("bstcow.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(72,60)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)


#############################################################################
#############################################################################
#3-Way Model: Human:Effluent(OST:H-e) vs Domestic(OST:C,E,D + BST:D) vs Wild(OST:B,W + BST:B)
#############################################################################
#############################################################################

##----------Linear Discriminant Analysis Set-Up
library(MASS)
ost<-read.csv("ost3way.csv")
ost.known<-ost[!is.na(ost$Type),2:118]
ost.known$Type[ost.known$Type==1]<-"Human"
ost.known$Type[ost.known$Type==2]<-"Domestic"
ost.known$Type[ost.known$Type==3]<-"Wild"
ost.known[,1]<-as.factor(ost.known[,1])
table(ost.known$Type)

##----------Graphically Surveying Data
source("multiDensityPlots.R")
multi.density.plots(ost.known[-1],ost.known[,1],2,5)
source("crossPlot.R")
cross.plot(ost.known[,-1],ost.known[,1],2,5)
source("GAPplot.R")
source("kFoldSplit.R")
o.split<-k.fold.split(ost.known[,1],4)
GAP.plot(ost.known[o.split==1,-1],ost.known[o.split==1,1])

##----------Linear Discriminant Analysis
ost.lda<-lda(Type~.,data=ost.known)
ost.lda.pred<-predict(ost.lda,dimen=4) #DA-Even Priors
(ost.lda.table<-table(ost.known$Type,ost.lda.pred$class))
(ost.lda.rcc<-diag(ost.lda.table)/apply(ost.lda.table,1,sum))
(overall.lda.rcc<-sum(diag(ost.lda.table))/sum(ost.lda.table))

ost.lda.cv<-lda(Type~.,data=ost.known,CV=T) #DA-Cross Validation
(ost.lda.cv.table<-table(ost.known$Type,ost.lda.cv$class))
(ost.lda.cv.rcc<-diag(ost.lda.cv.table)/apply(ost.lda.cv.table,1,sum))
(overall.lda.cv.rcc<-sum(diag(ost.lda.cv.table))/sum(ost.lda.cv.table))

ost.lda.pred.p<-predict(ost.lda,prior=c(0.33,0.34,0.33),dimen=4) #DA-Defined Priors
(ost.lda.table.p<-table(ost.known$Type,ost.lda.pred.p$class))
(ost.lda.rcc.p<-diag(ost.lda.table.p)/apply(ost.lda.table.p,1,sum))
(overall.lda.rcc.p<-sum(diag(ost.lda.table.p))/sum(ost.lda.table.p))

ost.lda.cv<-lda(Type~.,data=ost.known,prior=c(0.33,0.34,0.33),CV=T) #DA-Cross Validation w/Defined Priors
(ost.lda.cv.table<-table(ost.known$Type,ost.lda.cv$class))
(ost.lda.cv.rcc<-diag(ost.lda.cv.table)/apply(ost.lda.cv.table,1,sum))
(overall.lda.cv.rcc<-sum(diag(ost.lda.cv.table))/sum(ost.lda.cv.table))

##----------Random Forests
library(randomForest)
ost.rf<-read.csv("ost3way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Domestic"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])
```

```
table(ost.known.rf$Type)
ost.k.rf<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T) #RF
ost.k.rf$confusion
(ost.k.rf)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65))        #RF-
Stratified for sample sizes
ost.k.rf.s$confusion
(ost.k.rf.s)

##----------Set-up 80%/20% Validation
x<-read.csv("ost3way.csv")
x[,2]<-as.factor(x[,2])

#Takes 20% of Known Library out and test's it against the remaining 80% for both LDA and RF
#loop 100 times testing LDA and RF w/20% each
source("kFoldSplit.R"); library(MASS); library(randomForest)

lda.rcc<-rf.rcc<-matrix(0,100,3)
for (i in 1:100) {
        splitz<-k.fold.split(x[,2],3)  #  keeps proportions of each class
        train.set<-x[splitz!=1,]
        test.set<-x[splitz==1,]
        lda.model<-lda(Type~.,data=train.set[,-1],prior=c(0.34,0.33,0.33))
        lda.test<-predict(lda.model,test.set)
        lda.table<-table(test.set$Type,lda.test$class)
        lda.rcc[i,]<-diag(lda.table)/apply(lda.table,1,sum)
        rf.model<-randomForest(Type~.,data=train.set[,-1],strata=train.set$Type,sampsize=round(0.65*c(72,65,65)))
        rf.test<-predict(rf.model,test.set)
        rf.table<-table(test.set$Type,rf.test)
        rf.rcc[i,]<-diag(rf.table)/apply(rf.table,1,sum)
        print(i)
}

lo.pct<-function(x) {quantile(x,0.025)}; hi.pct<-function(x) {quantile(x,0.975)}
lda.pct.0.025<-apply(lda.rcc,2,lo.pct); lda.pct.0.975<-apply(lda.rcc,2,hi.pct); lda.mean<-apply(lda.rcc,2,mean)
rf.pct.0.025<-apply(rf.rcc,2,lo.pct); rf.pct.0.975<-apply(rf.rcc,2,hi.pct); rf.mean<-apply(rf.rcc,2,mean)
species<-c("Human","Domestic","Wild Animal")

plot(c(0,1),c(0,4),type="n",ylab=NA,xlab="means and 95% intervals for rcc",sub="red for lda, blue for rf")
for (j in 1:3) {
        lines(c(lda.pct.0.025[j],lda.pct.0.975[j]),rep(j-0.1,2),col="red",lty=2)
        text(lda.pct.0.025[j],j-0.1,"(",col="red");text(lda.pct.0.975[j],j-0.1,")",col="red")
        points(lda.mean[j],j-0.1,pch="*",col="red")
        lines(c(rf.pct.0.025[j],rf.pct.0.975[j]),rep(j+0.1,2),col="blue",lty=2)
        text(rf.pct.0.025[j],j+0.1,"(",col="blue");text(rf.pct.0.975[j],j+0.1,")",col="blue")
        points(rf.mean[j],j+0.1,pch="*",col="blue")
        text(lda.mean[j],j,species[j])
}

(overall.lda.rcc<-sum(diag(lda.table))/sum(lda.table)) #Computer ARCC for LDA
rf.model #Computer ARCC for RF (remember to subtract OOB error rate from 100% to get ARCC)

##----------This generates RF model 100 times and estimates the RCC ranges for each class.
rf.rcc.2<-matrix(0,100,3)
for (i in 1:100) {
        rf.model<-randomForest(Type~.,data=x[,-1],strata=x$Type,sampsize=c(72,65,65))
        rf.rcc.2[i,]<-1-rf.model$confusion[,4]
        print(i)
}

lo.pct<-function(x) {quantile(x,0.025)}; hi.pct<-function(x) {quantile(x,0.975)}
rf.pct.0.025<-apply(rf.rcc.2,2,lo.pct); rf.pct.0.975<-apply(rf.rcc.2,2,hi.pct); rf.mean<-apply(rf.rcc.2,2,mean)
species<-c("Human","Domestic","Wild Animal")

plot(c(0,1),c(0,4),type="n",ylab=NA,xlab="means +/- sd and 95% intervals for rcc",sub="red for lda, blue for rf")
for (j in 1:3) {
        lines(c(rf.pct.0.025[j],rf.pct.0.975[j]),rep(j,2),col="blue",lty=2)
        points(rf.mean[j],j,pch="*",col="blue")
        text(rf.pct.0.025[j],j,"(",col="blue");text(rf.pct.0.975[j],j,")",col="blue")
        print(paste(species[j],"=",signif(rf.mean[j],3),"+/-",signif(apply(rf.rcc.2,2,sd),3)))
        text(rf.mean[j],j+0.25,paste(species[j],"=",signif(rf.mean[j],3),"+/-",signif(sd(rf.rcc.2[,j]),3)))
}

##----------Using the 3-Way Model for Unknowns using LDA equal priors
library(MASS)
ost<-read.csv("ost3way.csv")
ost.known<-ost[!is.na(ost$Type),2:118]
ost.known$Type[ost.known$Type==1]<-"Human"
ost.known$Type[ost.known$Type==2]<-"Domestic"
```

```
ost.known$Type[ost.known$Type==3]<-"Wild"
ost.known[,1]<-as.factor(ost.known[,1])
table(ost.known$Type)

ost.u<-read.csv("unknowns.csv")
ost.unknown<-ost.u[!is.na(ost.u$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown<-ost.unknown[!is.na(ost.unknown[,1]),]
ost.unknown$Type[ost.unknown$Type==8]<-"Unknowns"
table(ost.unknown$Type)

ost.lda.three<-lda(Type~.,data=ost.known)
ost.plda.three<-predict(ost.lda.three,ost.unknown)
(ost.lda.table<-table(ost.unknown$Type,ost.plda.three$class))

##----------Using the 3-Way Model for Unknowns using RF stratified
library(randomForest)
ost.rf<-read.csv("ost3way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Domestic"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])
table(ost.known.rf$Type)

ost.u.rf<-read.csv("unknowns.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T) #RF
ost.u.rf<-predict(ost.k.rf,ost.unknown.rf)
table(ost.u.rf)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 3-Way Model for Unknowns - Wet Events using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost3way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Domestic"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-W.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 3-Way Model for Unknowns - Dry Events using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost3way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Domestic"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-D.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 3-Way Model for Unknowns - Dry Events Stations 499, 500, & 559 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost3way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
```

```
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Domestic"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-D-499-500-559.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 3-Way Model for Unknowns - Dry Events Stations 501 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost3way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Domestic"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-D-501.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 3-Way Model for Unknowns - Wet Events Stations 499, 500, & 559 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost3way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Domestic"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-W-499-500-559.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 3-Way Model for Unknowns - Dry Events Stations 198 & 501 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost3way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Domestic"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-W-198-501.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 3-Way Model for Human Unknowns for External Isolate Validation
library(randomForest)
ost.rf<-read.csv("ost3way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Domestic"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])
```

```
ost.u.rf<-read.csv("bsthuman.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 3-Way Model for Bovine Unknowns for External Isolate Validation
library(randomForest)
ost.rf<-read.csv("ost3way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Domestic"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("bstcow.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)


################################################################################
################################################################################
#4-Way Model: Human:Effluent(OST:H-e) vs Livestock(OST:C,E) vs Dog(OST/BST:D) vs Wild(OST:B,W + BST:B)
################################################################################
################################################################################

##----------Linear Discriminant Analysis Set-Up
library(MASS)
ost<-read.csv("ost4way.csv")
ost.known<-ost[!is.na(ost$Type),2:118]
ost.known$Type[ost.known$Type==1]<-"Human"
ost.known$Type[ost.known$Type==2]<-"Livestock"
ost.known$Type[ost.known$Type==3]<-"Dog"
ost.known$Type[ost.known$Type==4]<-"Wild"
ost.known[,1]<-as.factor(ost.known[,1])
table(ost.known$Type)

##----------Graphically Surveying Data
source("multiDensityPlots.R")
multi.density.plots(ost.known[-1],ost.known[,1],2,5)
source("crossPlot.R")
cross.plot(ost.known[,-1],ost.known[,1],2,5)
source("GAPplot.R")
source("kFoldSplit.R")
o.split<-k.fold.split(ost.known[,1],4)
GAP.plot(ost.known[o.split==1,-1],ost.known[o.split==1,1])

##----------Linear Discriminant Analysis
ost.lda<-lda(Type~.,data=ost.known)
ost.lda.pred<-predict(ost.lda,dimen=4) #DA-Even Priors
(ost.lda.table<-table(ost.known$Type,ost.lda.pred$class))
(ost.lda.rcc<-diag(ost.lda.table)/apply(ost.lda.table,1,sum))
(overall.lda.rcc<-sum(diag(ost.lda.table))/sum(ost.lda.table))

ost.lda.cv<-lda(Type~.,data=ost.known,CV=T) #DA-Cross Validation
(ost.lda.cv.table<-table(ost.known$Type,ost.lda.cv$class))
(ost.lda.cv.rcc<-diag(ost.lda.cv.table)/apply(ost.lda.cv.table,1,sum))
(overall.lda.cv.rcc<-sum(diag(ost.lda.cv.table))/sum(ost.lda.cv.table))

ost.lda.pred.p<-predict(ost.lda,prior=c(0.24,0.24,0.28,0.24),dimen=4) #DA-Defined Priors
(ost.lda.table.p<-table(ost.known$Type,ost.lda.pred.p$class))
(ost.lda.rcc.p<-diag(ost.lda.table.p)/apply(ost.lda.table.p,1,sum))
(overall.lda.rcc.p<-sum(diag(ost.lda.table.p))/sum(ost.lda.table.p))

ost.lda.cv<-lda(Type~.,data=ost.known,prior=c(0.24,0.24,0.28,0.24),CV=T) #DA-Cross Validation w/Defined Priors
(ost.lda.cv.table<-table(ost.known$Type,ost.lda.cv$class))
(ost.lda.cv.rcc<-diag(ost.lda.cv.table)/apply(ost.lda.cv.table,1,sum))
(overall.lda.cv.rcc<-sum(diag(ost.lda.cv.table))/sum(ost.lda.cv.table))
```

```
##----------Random Forests
library(randomForest)
ost.rf<-read.csv("ost4way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

table(ost.known.rf$Type)
ost.k.rf<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T) #RF
ost.k.rf$confusion
(ost.k.rf)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65,70))     #RF-
Stratified for sample sizes
ost.k.rf.s$confusion
(ost.k.rf.s)

##----------Set-up 80%/20% Validation
x<-read.csv("ost4way.csv")
x[,2]<-as.factor(x[,2])

#Takes 20% of Known Library out and test's it against the remaining 80% for both LDA and RF
#loop 100 times testing LDA and RF w/20% each
source("kFoldSplit.R"); library(MASS); library(randomForest)

lda.rcc<-rf.rcc<-matrix(0,100,4)
for (i in 1:100) {
        splitz<-k.fold.split(x[,2],4)  #  keeps proportions of each class
        train.set<-x[splitz!=1,]
        test.set<-x[splitz==1,]
        lda.model<-lda(Type~.,data=train.set[,-1],prior=c(0.24,0.28,0.24,0.24))
        lda.test<-predict(lda.model,test.set)
        lda.table<-table(test.set$Type,lda.test$class)
        lda.rcc[i,]<-diag(lda.table)/apply(lda.table,1,sum)
        rf.model<-randomForest(Type~.,data=train.set[,-1],strata=train.set$Type,sampsize=round(0.58*c(72,65,65,70)))
        rf.test<-predict(rf.model,test.set)
        rf.table<-table(test.set$Type,rf.test)
        rf.rcc[i,]<-diag(rf.table)/apply(rf.table,1,sum)
        print(i)
}

lo.pct<-function(x) {quantile(x,0.025)}; hi.pct<-function(x) {quantile(x,0.975)}
lda.pct.0.025<-apply(lda.rcc,2,lo.pct); lda.pct.0.975<-apply(lda.rcc,2,hi.pct); lda.mean<-apply(lda.rcc,2,mean)
rf.pct.0.025<-apply(rf.rcc,2,lo.pct); rf.pct.0.975<-apply(rf.rcc,2,hi.pct); rf.mean<-apply(rf.rcc,2,mean)
species<-c("Human","Livestock","Dog","Wild Animal")

plot(c(0,1),c(0,5),type="n",ylab=NA,xlab="means and 95% intervals for rcc",sub="red for lda, blue for rf")
for (j in 1:4) {
        lines(c(lda.pct.0.025[j],lda.pct.0.975[j]),rep(j-0.1,2),col="red",lty=2)
        text(lda.pct.0.025[j],j-0.1,"(",col="red");text(lda.pct.0.975[j],j-0.1,")",col="red")
        points(lda.mean[j],j-0.1,pch="*",col="red")
        lines(c(rf.pct.0.025[j],rf.pct.0.975[j]),rep(j+0.1,2),col="blue",lty=2)
        text(rf.pct.0.025[j],j+0.1,"(",col="blue");text(rf.pct.0.975[j],j+0.1,")",col="blue")
        points(rf.mean[j],j+0.1,pch="*",col="blue")
        text(lda.mean[j],j,species[j])
}

(overall.lda.rcc<-sum(diag(lda.table))/sum(lda.table)) #Computer ARCC for LDA
rf.model #Computer ARCC for RF (remember to subtract OOB error rate from 100% to get ARCC)

##----------This generates RF model 100 times and estimates the RCC ranges for each class.
rf.rcc.2<-matrix(0,100,4)
for (i in 1:100) {
        rf.model<-randomForest(Type~.,data=x[,-1],strata=x$Type,sampsize=c(72,65,65,70))
        rf.rcc.2[i,]<-1-rf.model$confusion[,5]
        print(i)
}

lo.pct<-function(x) {quantile(x,0.025)}; hi.pct<-function(x) {quantile(x,0.975)}
rf.pct.0.025<-apply(rf.rcc.2,2,lo.pct); rf.pct.0.975<-apply(rf.rcc.2,2,hi.pct); rf.mean<-apply(rf.rcc.2,2,mean)
species<-c("Human","Livestock","Dog","Wild Animal")

plot(c(0,1),c(0,5),type="n",ylab=NA,xlab="means +/- sd and 95% intervals for rcc",sub="red for lda, blue for rf")
for (j in 1:4) {
        lines(c(rf.pct.0.025[j],rf.pct.0.975[j]),rep(j,2),col="blue",lty=2)
        points(rf.mean[j],j,pch="*",col="blue")
        text(rf.pct.0.025[j],j,"(",col="blue");text(rf.pct.0.975[j],j,")",col="blue")
```

```
            print(paste(species[j],"=",signif(rf.mean[j],3),"+/-",signif(apply(rf.rcc.2,2,sd),3)))
            text(rf.mean[j],j+0.25,paste(species[j],"=",signif(rf.mean[j],3),"+/-",signif(sd(rf.rcc.2[,j]),3)))
}

##----------Using the 4-Way Model for Unknowns using LDA equal priors
library(MASS)
ost<-read.csv("ost4way.csv")
ost.known<-ost[!is.na(ost$Type),2:118]
ost.known$Type[ost.known$Type==1]<-"Human"
ost.known$Type[ost.known$Type==2]<-"Livestock"
ost.known$Type[ost.known$Type==3]<-"Dog"
ost.known$Type[ost.known$Type==4]<-"Wild"
ost.known[,1]<-as.factor(ost.known[,1])
table(ost.known$Type)

ost.u<-read.csv("unknowns.csv")
ost.unknown<-ost.u[!is.na(ost.u$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown<-ost.unknown[!is.na(ost.unknown[,1]),]
ost.unknown$Type[ost.unknown$Type==8]<-"Unknowns"
table(ost.unknown$Type)

ost.lda.four<-lda(Type~.,data=ost.known)
ost.plda.four<-predict(ost.lda.four,ost.unknown)
(ost.lda.table<-table(ost.unknown$Type,ost.plda.four$class))

##----------Using the 4-Way Model for Unknowns using RF-stratified
library(randomForest)
ost.rf<-read.csv("ost4way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])
table(ost.known.rf$Type)

ost.u.rf<-read.csv("unknowns.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T) #RF
ost.u.rf<-predict(ost.k.rf,ost.unknown.rf)
table(ost.u.rf)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65,70)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 4-Way Model for Unknowns - Wet Events using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost4way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-W.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65,70)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 4-Way Model for Unknowns - Dry Events using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost4way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])
```

```
ost.u.rf<-read.csv("U-D.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65,70)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 4-Way Model for Unknowns - Dry Events Stations 499, 500, & 559 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost4way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-D-499-500-559.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65,70)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 4-Way Model for Unknowns - Dry Events Stations 501 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost4way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-D-501.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65,70)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 4-Way Model for Unknowns - Wet Events Stations 499, 500, & 559 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost4way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-W-499-500-559.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65,70)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 4-Way Model for Unknowns - Dry Events Stations 198 & 501 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost4way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])
```

```
ost.u.rf<-read.csv("U-W-198-501.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65,70)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 4-Way Model for Human Unknowns for External Isolate Validation
library(randomForest)
ost.rf<-read.csv("ost4way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("bsthuman.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65,70)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 4-Way Model for Bovine Unknowns for External Isolate Validation
library(randomForest)
ost.rf<-read.csv("ost4way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Wild"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("bstcow.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,72,65,70)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)


#############################################################################
#############################################################################
#5-Way Model: Human:Effluent(OST:H-e) vs Livestock(OST:C,E) vs Dog(OST/BST:D) vs Seagull(BST:B) vs Wildlife(OST:B,W)
#############################################################################
#############################################################################

##----------Linear Discriminant Analysis Set-Up
library(MASS)
ost<-read.csv("ost5way.csv")
ost.known<-ost[!is.na(ost$Type),2:118]
ost.known$Type[ost.known$Type==1]<-"Human"
ost.known$Type[ost.known$Type==2]<-"Livestock"
ost.known$Type[ost.known$Type==3]<-"Dog"
ost.known$Type[ost.known$Type==4]<-"Seagull"
ost.known$Type[ost.known$Type==5]<-"Wildlife(Avian/Non)"
ost.known[,1]<-as.factor(ost.known[,1])
table(ost.known$Type)

##----------Graphically Surveying Data
source("multiDensityPlots.R")
multi.density.plots(ost.known[-1],ost.known[,1],2,5)
source("crossPlot.R")
cross.plot(ost.known[,-1],ost.known[,1],2,5)
source("GAPplot.R")
source("kFoldSplit.R")
o.split<-k.fold.split(ost.known[,1],4)
GAP.plot(ost.known[o.split==1,-1],ost.known[o.split==1,1])

##----------Linear Discriminant Analysis
```

```
ost.lda<-lda(Type~.,data=ost.known)
ost.lda.pred<-predict(ost.lda,dimen=4) #DA-Even Priors
(ost.lda.table<-table(ost.known$Type,ost.lda.pred$class))
(ost.lda.rcc<-diag(ost.lda.table)/apply(ost.lda.table,1,sum))
(overall.lda.rcc<-sum(diag(ost.lda.table))/sum(ost.lda.table))

ost.lda.cv<-lda(Type~.,data=ost.known,CV=T) #DA-Cross Validation
(ost.lda.cv.table<-table(ost.known$Type,ost.lda.cv$class))
(ost.lda.cv.rcc<-diag(ost.lda.cv.table)/apply(ost.lda.cv.table,1,sum))
(overall.lda.cv.rcc<-sum(diag(ost.lda.cv.table))/sum(ost.lda.cv.table))

ost.lda.pred.p<-predict(ost.lda,prior=c(0.18,0.27,0.19,0.18,0.18),dimen=4) #DA-Defined Priors
(ost.lda.table.p<-table(ost.known$Type,ost.lda.pred.p$class))
(ost.lda.rcc.p<-diag(ost.lda.table.p)/apply(ost.lda.table.p,1,sum))
(overall.lda.rcc.p<-sum(diag(ost.lda.table.p))/sum(ost.lda.table.p))

ost.lda.cv<-lda(Type~.,data=ost.known,prior=c(0.18,0.27,0.19,0.18,0.18),CV=T) #DA-Cross Validation w/Defined Priors
(ost.lda.cv.table<-table(ost.known$Type,ost.lda.cv$class))
(ost.lda.cv.rcc<-diag(ost.lda.cv.table)/apply(ost.lda.cv.table,1,sum))
(overall.lda.cv.rcc<-sum(diag(ost.lda.cv.table))/sum(ost.lda.cv.table))

##----------Random Forests
library(randomForest)
ost.rf<-read.csv("ost5way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Wildlife(Avian/Non)"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

table(ost.known.rf$Type)
ost.k.rf<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T) #RF
ost.k.rf$confusion
(ost.k.rf)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(60,72,67,65,65))
#RF-Stratified for sample sizes
ost.k.rf.s$confusion
(ost.k.rf.s)

##----------Set-up for 80%/20% Validation
x<-read.csv("ost5way.csv")
x[,2]<-as.factor(x[,2])

#Takes 20% of Known Library out and test's it against the remaining 80% for both LDA and RF
#loop 100 times testing LDA and RF w/20% each
source("kFoldSplit.R"); library(MASS); library(randomForest)

lda.rcc<-rf.rcc<-matrix(0,100,5)
for (i in 1:100) {
        splitz<-k.fold.split(x[,2],5)  #  keeps proportions of each class
        train.set<-x[splitz!=1,]
        test.set<-x[splitz==1,]
        lda.model<-lda(Type~.,data=train.set[,-1],prior=c(0.27,0.19,0.18,0.18,0.18))
        lda.test<-predict(lda.model,test.set)
        lda.table<-table(test.set$Type,lda.test$class)
        lda.rcc[i,]<-diag(lda.table)/apply(lda.table,1,sum)
        rf.model<-randomForest(Type~.,data=train.set[,-1],strata=train.set$Type,sampsize=round(0.78*c(72,67,60,65,65)))
        #tofuturegradstudentsreadingthis,getoutwhileyoucan
        rf.test<-predict(rf.model,test.set)
        rf.table<-table(test.set$Type,rf.test)
        rf.rcc[i,]<-diag(rf.table)/apply(rf.table,1,sum)
        print(i)
}

lo.pct<-function(x) {quantile(x,0.025)}; hi.pct<-function(x) {quantile(x,0.975)}
lda.pct.0.025<-apply(lda.rcc,2,lo.pct); lda.pct.0.975<-apply(lda.rcc,2,hi.pct); lda.mean<-apply(lda.rcc,2,mean)
rf.pct.0.025<-apply(rf.rcc,2,lo.pct); rf.pct.0.975<-apply(rf.rcc,2,hi.pct); rf.mean<-apply(rf.rcc,2,mean)
species<-c("Human","Livestock","Dog","Seagull","Wildlife(Avian/Non)")

plot(c(0,1),c(0,6),type="n",ylab=NA,xlab="means and 95% intervals for rcc",sub="red for lda, blue for rf")
for (j in 1:5) {
        lines(c(lda.pct.0.025[j],lda.pct.0.975[j]),rep(j-0.1,2),col="red",lty=2)
        text(lda.pct.0.025[j],j-0.1,"(",col="red");text(lda.pct.0.975[j],j-0.1,")",col="red")
        points(lda.mean[j],j-0.1,pch="*",col="red")
        lines(c(rf.pct.0.025[j],rf.pct.0.975[j]),rep(j+0.1,2),col="blue",lty=2)
        text(rf.pct.0.025[j],j+0.1,"(",col="blue");text(rf.pct.0.975[j],j+0.1,")",col="blue")
        points(rf.mean[j],j+0.1,pch="*",col="blue")
```

```
		text(lda.mean[j],j,species[j])
}

(overall.lda.rcc<-sum(diag(lda.table))/sum(lda.table)) #Computer ARCC for LDA
rf.model #Computer ARCC for RF (remember to subtract OOB error rate from 100% to get ARCC)

##----------This generates RF model 100 times and estimates the RCC ranges for each class.
rf.rcc.2<-matrix(0,100,5)
for (i in 1:100) {
		rf.model<-randomForest(Type~.,data=x[,-1],strata=x$Type,sampsize=c(72,67,60,65,65))
		rf.rcc.2[i,]<-1-rf.model$confusion[,6]
		print(i)
}

lo.pct<-function(x) {quantile(x,0.025)}; hi.pct<-function(x) {quantile(x,0.975)}
rf.pct.0.025<-apply(rf.rcc.2,2,lo.pct); rf.pct.0.975<-apply(rf.rcc.2,2,hi.pct); rf.mean<-apply(rf.rcc.2,2,mean)
species<-c("Human","Livestock","Dog","Seagull","Wildlife(Avian/Non)")

plot(c(0,1),c(0,6),type="n",ylab=NA,xlab="means +/- sd and 95% intervals for rcc",sub="red for lda, blue for rf")
for (j in 1:5) {
		lines(c(rf.pct.0.025[j],rf.pct.0.975[j]),rep(j,2),col="blue",lty=2)
		points(rf.mean[j],j,pch="*",col="blue")
		text(rf.pct.0.025[j],j,"(",col="blue");text(rf.pct.0.975[j],j,")",col="blue")
		print(paste(species[j],"=",signif(rf.mean[j],3),"+/-",signif(apply(rf.rcc.2,2,sd),3)))
		text(rf.mean[j],j+0.25,paste(species[j],"=",signif(rf.mean[j],3),"+/-",signif(sd(rf.rcc.2[,j]),3)))
}

##----------Using the 5-Way Model for Unknowns using LDA equal priors
library(MASS)
ost<-read.csv("ost5way.csv")
ost.known<-ost[!is.na(ost$Type),2:118]
ost.known$Type[ost.known$Type==1]<-"Human"
ost.known$Type[ost.known$Type==2]<-"Livestock"
ost.known$Type[ost.known$Type==3]<-"Dog"
ost.known$Type[ost.known$Type==4]<-"Seagull"
ost.known$Type[ost.known$Type==5]<-"Wildlife(Avian/Non)"
ost.known[,1]<-as.factor(ost.known[,1])
table(ost.known$Type)

ost.u<-read.csv("unknowns.csv")
ost.unknown<-ost.u[!is.na(ost.u$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown<-ost.unknown[!is.na(ost.unknown[,1]),]
ost.unknown$Type[ost.unknown$Type==8]<-"Unknowns"
table(ost.unknown$Type)

ost.lda.five<-lda(Type~.,data=ost.known)
ost.plda.five<-predict(ost.lda.five,ost.unknown)
(ost.lda.table<-table(ost.unknown$Type,ost.plda.five$class))

##----------Using the 5-Way Model for Unknowns using RF stratified
library(randomForest)
ost.rf<-read.csv("ost5way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Wildlife(Avian/Non)"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])
table(ost.known.rf$Type)

ost.u.rf<-read.csv("unknowns.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T) #RF
ost.u.rf<-predict(ost.k.rf,ost.unknown.rf)
table(ost.u.rf)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(60,72,67,65,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 5-Way Model for Unknowns - Wet Events using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost5way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
```

```
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Wildlife(Avian/Non)"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-W.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
#nooneisgoingtoreadthis
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(60,72,67,65,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 5-Way Model for Unknowns - Dry Events using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost5way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Wildlife(Avian/Non)"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-D.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(60,72,67,65,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 5-Way Model for Unknowns - Dry Events Stations 499, 500, & 559 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost5way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Wildlife(Avian/Non)"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-D-499-500-559.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(60,72,67,65,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 5-Way Model for Unknowns - Dry Events Stations 501 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost5way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Wildlife(Avian/Non)"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-D-501.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(60,72,67,65,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)
```

```
##----------Using the 5-Way Model for Unknowns - Wet Events Stations 499, 500, & 559 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost5way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Wildlife(Avian/Non)"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-W-499-500-559.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(60,72,67,65,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 5-Way Model for Unknowns - Dry Events Stations 198 & 501 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost5way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Wildlife(Avian/Non)"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-W-198-501.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(60,72,67,65,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 5-Way Model for Human Unknowns for External Isolate Validation
library(randomForest)
ost.rf<-read.csv("ost5way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Wildlife(Avian/Non)"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("bsthuman.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(60,72,67,65,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 5-Way Model for Bovine Unknowns for External Isolate Validation
library(randomForest)
ost.rf<-read.csv("ost5way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Livestock"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Wildlife(Avian/Non)"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("bstcow.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)
```

```
ost.k.rf.s<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(60,72,67,65,65)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)


#############################################################################
#############################################################################
#7-Way Model: Human:Effluent(OST:H-e) vs Cow(OST:C) vs Horse(OST:E) vs Dog(OST/BST:D) vs Seagull(BST:B) vs Bird(OST:B) vs Wildlife(OST:W)
#############################################################################
#############################################################################

##----------Linear Discriminant Analysis Set-Up
library(MASS)
ost<-read.csv("ost7way.csv")
ost.known<-ost[!is.na(ost$Type),2:118]
ost.known$Type[ost.known$Type==1]<-"Human"
ost.known$Type[ost.known$Type==2]<-"Cow"
ost.known$Type[ost.known$Type==3]<-"Horse"
ost.known$Type[ost.known$Type==4]<-"Dog"
ost.known$Type[ost.known$Type==5]<-"Seagull"
ost.known$Type[ost.known$Type==6]<-"OtherBird"
ost.known$Type[ost.known$Type==7]<-"Wildlife"
ost.known[,1]<-as.factor(ost.known[,1])
table(ost.known$Type)

##----------Graphically Surveying Data
source("multiDensityPlots.R")
multi.density.plots(ost.known[-1],ost.known[,1],2,5)
source("crossPlot.R")
cross.plot(ost.known[,-1],ost.known[,1],2,5)
source("GAPplot.R")
source("kFoldSplit.R")
o.split<-k.fold.split(ost.known[,1],4)
GAP.plot(ost.known[o.split==1,-1],ost.known[o.split==1,1])

##----------Linear Discriminant Analysis
ost.lda<-lda(Type~.,data=ost.known)
ost.lda.pred<-predict(ost.lda,dimen=4) #DA-Even Priors
(ost.lda.table<-table(ost.known$Type,ost.lda.pred$class))
(ost.lda.rcc<-diag(ost.lda.table)/apply(ost.lda.table,1,sum))
(overall.lda.rcc<-sum(diag(ost.lda.table))/sum(ost.lda.table))

ost.lda.cv<-lda(Type~.,data=ost.known,CV=T) #DA-Cross Validation
(ost.lda.cv.table<-table(ost.known$Type,ost.lda.cv$class))
(ost.lda.cv.rcc<-diag(ost.lda.cv.table)/apply(ost.lda.cv.table,1,sum))
(overall.lda.cv.rcc<-sum(diag(ost.lda.cv.table))/sum(ost.lda.cv.table))

ost.lda.pred.p<-predict(ost.lda,prior=c(0.13,0.13,0.17,0.15,0.15,0.13,0.14),dimen=4) #DA-Defined Priors
(ost.lda.table.p<-table(ost.known$Type,ost.lda.pred.p$class))
(ost.lda.rcc.p<-diag(ost.lda.table.p)/apply(ost.lda.table.p,1,sum))
(overall.lda.rcc.p<-sum(diag(ost.lda.table.p))/sum(ost.lda.table.p))

ost.lda.cv<-lda(Type~.,data=ost.known,prior=c(0.13,0.13,0.17,0.15,0.15,0.13,0.14),CV=T) #DA-Cross Validation w/Defined Priors
(ost.lda.cv.table<-table(ost.known$Type,ost.lda.cv$class))
(ost.lda.cv.rcc<-diag(ost.lda.cv.table)/apply(ost.lda.cv.table,1,sum))
(overall.lda.cv.rcc<-sum(diag(ost.lda.cv.table))/sum(ost.lda.cv.table))

##----------Random Forests
library(randomForest)
ost.rf<-read.csv("ost7way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Cow"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Horse"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==6]<-"OtherBird"
ost.known.rf$Type[ost.known.rf$Type==7]<-"Wildlife"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

table(ost.known.rf$Type)
ost.k.rf<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T) #RF
ost.k.rf$confusion
(ost.k.rf)

ost.k.rf.s<-
randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,65,92,72,90,65,90))      #RF-Stratified for sample sizes
ost.k.rf.s$confusion
```

```
(ost.k.rf.s)

##----------Set-up for 80%/20% validation
x<-read.csv("ost7way.csv")
x[,2]<-as.factor(x[,2])

#Takes 20% of Known Library out and test's it against the remaining 80% for both LDA and RF
#loop 100 times testing LDA and RF w/20% each
source("kFoldSplit.R"); library(MASS); library(randomForest)

lda.rcc<-rf.rcc<-matrix(0,100,7)
for (i in 1:100) {
        splitz<-k.fold.split(x[,2],5)  #  keeps proportions of each class
        train.set<-x[splitz!=1,]
        test.set<-x[splitz==1,]
        lda.model<-lda(Type~.,data=train.set[,-1],prior=c(0.15,0.13,0.17,0.13,0.13,0.15,0.14))
        lda.test<-predict(lda.model,test.set)
        lda.table<-table(test.set$Type,lda.test$class)
        lda.rcc[i,]<-diag(lda.table)/apply(lda.table,1,sum)
        rf.model<-randomForest(Type~.,data=train.set[,-1],strata=train.set$Type,sampsize=round(0.78*c(72,65,92,65,65,90,90)))
        rf.test<-predict(rf.model,test.set)
        rf.table<-table(test.set$Type,rf.test)
        rf.rcc[i,]<-diag(rf.table)/apply(rf.table,1,sum)
        print(i)
}

lo.pct<-function(x) {quantile(x,0.025)}; hi.pct<-function(x) {quantile(x,0.975)}
lda.pct.0.025<-apply(lda.rcc,2,lo.pct); lda.pct.0.975<-apply(lda.rcc,2,hi.pct); lda.mean<-apply(lda.rcc,2,mean)
rf.pct.0.025<-apply(rf.rcc,2,lo.pct); rf.pct.0.975<-apply(rf.rcc,2,hi.pct); rf.mean<-apply(rf.rcc,2,mean)
species<-c("Human","Cow","Horse","Dog","Seagull","OtherBird","Wildlife")

plot(c(0,1),c(0,8),type="n",ylab=NA,xlab="means and 95% intervals for rcc",sub="red for lda, blue for rf")
for (j in 1:7) {
        lines(c(lda.pct.0.025[j],lda.pct.0.975[j]),rep(j-0.1,2),col="red",lty=2)
        text(lda.pct.0.025[j],j-0.1,"(",col="red");text(lda.pct.0.975[j],j-0.1,")",col="red")
        points(lda.mean[j],j-0.1,pch="*",col="red")
        lines(c(rf.pct.0.025[j],rf.pct.0.975[j]),rep(j+0.1,2),col="blue",lty=2)
        text(rf.pct.0.025[j],j+0.1,"(",col="blue");text(rf.pct.0.975[j],j+0.1,")",col="blue")
        points(rf.mean[j],j+0.1,pch="*",col="blue")
        text(lda.mean[j],j,species[j])
}

(overall.lda.rcc<-sum(diag(lda.table))/sum(lda.table)) #Computer ARCC for LDA
rf.model #Computer ARCC for RF (remember to subtract OOB error rate from 100% to get ARCC)

##----------This generates RF model 100 times and estimates the RCC ranges for each class.
rf.rcc.2<-matrix(0,100,7)
for (i in 1:100) {
        rf.model<-randomForest(Type~.,data=x[,-1],strata=x$Type,sampsize=c(72,65,92,65,65,90,90))
        rf.rcc.2[i,]<-1-rf.model$confusion[,8]
        print(i)
}

lo.pct<-function(x) {quantile(x,0.025)}; hi.pct<-function(x) {quantile(x,0.975)}
rf.pct.0.025<-apply(rf.rcc.2,2,lo.pct); rf.pct.0.975<-apply(rf.rcc.2,2,hi.pct); rf.mean<-apply(rf.rcc.2,2,mean)
species<-c("Human","Cow","Horse","Dog","Seagull","OtherBird","Wildlife")

plot(c(0,1),c(0,8),type="n",ylab=NA,xlab="means +/- sd and 95% intervals for rcc",sub="red for lda, blue for rf")
for (j in 1:7) {
        lines(c(rf.pct.0.025[j],rf.pct.0.975[j]),rep(j,2),col="blue",lty=2)
        points(rf.mean[j],j,pch="*",col="blue")
        text(rf.pct.0.025[j],j,"(",col="blue");text(rf.pct.0.975[j],j,")",col="blue")
        print(paste(species[j],"=",signif(rf.mean[j],3),"+/-",signif(apply(rf.rcc.2,2,sd),3)))
        text(rf.mean[j],j+0.25,paste(species[j],"=",signif(rf.mean[j],3),"+/-",signif(sd(rf.rcc.2[,j]),3)))
}

##----------Using the 7-Way Model for Unknowns using LDA equal priors
library(MASS)
ost<-read.csv("ost7way.csv")
ost.known<-ost[!is.na(ost$Type),2:118]
ost.known$Type[ost.known$Type==1]<-"Human"
ost.known$Type[ost.known$Type==2]<-"Cow"
ost.known$Type[ost.known$Type==3]<-"Horse"
ost.known$Type[ost.known$Type==4]<-"Dog"
ost.known$Type[ost.known$Type==5]<-"Seagull"
ost.known$Type[ost.known$Type==6]<-"OtherBird"
ost.known$Type[ost.known$Type==7]<-"Wildlife"
ost.known[,1]<-as.factor(ost.known[,1])
table(ost.known$Type)
```

```
ost.u<-read.csv("unknowns.csv")
ost.unknown<-ost.u[!is.na(ost.u$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown<-ost.unknown[!is.na(ost.unknown[,1]),]
ost.unknown$Type[ost.unknown$Type==8]<-"Unknowns"
table(ost.unknown$Type)

ost.lda.seven<-lda(Type~.,data=ost.known)
ost.plda.seven<-predict(ost.lda.seven,ost.unknown)
(ost.lda.table<-table(ost.unknown$Type,ost.plda.seven$class))

##----------Using the 7-Way Model for Unknowns using RF stratified
library(randomForest)
ost.rf<-read.csv("ost7way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Cow"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Horse"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==6]<-"OtherBird"
ost.known.rf$Type[ost.known.rf$Type==7]<-"Wildlife"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])
table(ost.known.rf$Type)

ost.u.rf<-read.csv("unknowns.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf<-randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T) #RF
ost.u.rf<-predict(ost.k.rf,ost.unknown.rf)
table(ost.u.rf)

ost.k.rf.s<-
randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,65,92,72,90,65,90)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 7-Way Model for Unknowns - Wet Events using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost7way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Cow"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Horse"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==6]<-"OtherBird"
ost.known.rf$Type[ost.known.rf$Type==7]<-"Wildlife"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-W.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-
randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,65,92,72,90,65,90)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 7-Way Model for Unknowns - Dry Events using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost7way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Cow"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Horse"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==6]<-"OtherBird"
ost.known.rf$Type[ost.known.rf$Type==7]<-"Wildlife"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-D.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
```

```
table(ost.unknown.rf$Type)

ost.k.rf.s<-
randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,65,92,72,90,65,90)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 7-Way Model for Unknowns - Dry Events Stations 499, 500, & 559 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost7way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Cow"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Horse"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==6]<-"OtherBird"
ost.known.rf$Type[ost.known.rf$Type==7]<-"Wildlife"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-D-499-500-559.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-
randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,65,92,72,90,65,90)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 7-Way Model for Unknowns - Dry Events Stations 501 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost7way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Cow"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Horse"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==6]<-"OtherBird"
ost.known.rf$Type[ost.known.rf$Type==7]<-"Wildlife"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-D-501.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-
randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,65,92,72,90,65,90)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 7-Way Model for Unknowns - Wet Events Stations 499, 500, & 559 using RF-Stratified
library(randomForest)
ost.rf<-read.csv("ost7way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Cow"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Horse"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==6]<-"OtherBird"
ost.known.rf$Type[ost.known.rf$Type==7]<-"Wildlife"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-W-499-500-559.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-
randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,65,92,72,90,65,90)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 7-Way Model for Unknowns - Dry Events Stations 198 & 501 using RF-Stratified
```

```
library(randomForest)
ost.rf<-read.csv("ost7way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Cow"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Horse"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==6]<-"OtherBird"
ost.known.rf$Type[ost.known.rf$Type==7]<-"Wildlife"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("U-W-198-501.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-
randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,65,92,72,90,65,90)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 7-Way Model for Human Unknowns for External Isolate Validation
library(randomForest)
ost.rf<-read.csv("ost7way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Cow"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Horse"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==6]<-"OtherBird"
ost.known.rf$Type[ost.known.rf$Type==7]<-"Wildlife"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("bsthuman.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-
randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,65,92,72,90,65,90)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##----------Using the 7-Way Model for Bovine Unknowns for External Isolate Validation
library(randomForest)
ost.rf<-read.csv("ost7way.csv")
ost.known.rf<-ost.rf[!is.na(ost.rf$Type),2:118]
ost.known.rf$Type[ost.known.rf$Type==1]<-"Human"
ost.known.rf$Type[ost.known.rf$Type==2]<-"Cow"
ost.known.rf$Type[ost.known.rf$Type==3]<-"Horse"
ost.known.rf$Type[ost.known.rf$Type==4]<-"Dog"
ost.known.rf$Type[ost.known.rf$Type==5]<-"Seagull"
ost.known.rf$Type[ost.known.rf$Type==6]<-"OtherBird"
ost.known.rf$Type[ost.known.rf$Type==7]<-"Wildlife"
ost.known.rf[,1]<-as.factor(ost.known.rf[,1])

ost.u.rf<-read.csv("bstbovine.csv")
ost.unknown.rf<-ost.u.rf[!is.na(ost.u.rf$Type),2:118]  #  Create a separate table with the unknown isolates
ost.unknown.rf<-ost.unknown.rf[!is.na(ost.unknown.rf[,1]),]
ost.unknown.rf$Type[ost.unknown.rf$Type==8]<-"Unknowns"
table(ost.unknown.rf$Type)

ost.k.rf.s<-
randomForest(Type~.,data=ost.known.rf,do.trace=50,importance=T,strata=ost.known.rf$Type,sampsize=c(65,65,92,72,90,65,90)) #RF
ost.u.rf.s<-predict(ost.k.rf.s,ost.unknown.rf)
table(ost.u.rf.s)

##---------LDA 7-way Variables of Importance
library(MASS)
ost<-read.csv("ost7way.csv")
x<-ost[!is.na(ost$Type),2:118]
x$Type[x$Type==1]<-"Human"
x$Type[x$Type==2]<-"Cow"
x$Type[x$Type==3]<-"Horse"
x$Type[x$Type==4]<-"Dog"
x$Type[x$Type==5]<-"Seagull"
```

```
x$Type[x$Type==6]<-"OtherBird"
x$Type[x$Type==7]<-"Wildlife"
x[,1]<-as.factor(x[,1])
table(x$Type)

ost.lda.x<-lda(Type~.,data=x)
ost.lda.pred.x<-predict(ost.lda.x,dimen=4) #DA-Even Priors
(ost.lda.table.x<-table(x$Type,ost.lda.pred.x$class))
(x.lda.rcc<-diag(ost.lda.table.x)/apply(ost.lda.table.x,1,sum))
(overall.lda.rcc.x<-sum(diag(ost.lda.table.x))/sum(ost.lda.table.x))

reduction<-matrix(0,7,117)
colnames(reduction)<-colnames(x)[2:118]
rownames(reduction)<-levels(x$Type)
print("Overall")
print(ost.lda.table.x)
for (j in 2:21) {
        x.lda.j<-lda(Type~.,data=x[,-j],prior=rep(1/7,7))
        x.lda.j.pred<-predict(x.lda.j)
        rcc.j<-table(x$Type,x.lda.j.pred$class)
        print(colnames(x)[j])
        print(rcc.j)
        reduction[,j-1]<-diag(rcc.j)/apply(rcc.j,1,sum)-x.lda.rcc
}
reduction

F.vals<-p.vals<-1:117; names(F.vals)<-names(p.vals)<-colnames(x)[2:118]
for (j in 2:118) {
        aov.j<-anova(aov(x[,j]~x[,1]))
        F.vals[j-1]<-aov.j[1,4]
        p.vals[j-1]<-aov.j[1,5]
}

sort(F.vals)
sort(p.vals)


#############################################################################
#Citations
#############################################################################

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#R: A language and environment for statistical computing.
#R Foundation for Statistical Computing, Vienna, Austria.
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#ISBN 3-900051-07-0
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#############################################################################
#END
#############################################################################
```

Appendix E
Animal Isolate Information

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	Genus/Species
BST-B-2-1	Laughing Gull	Malaquite Beach	5/22/2003	7/20/2003	Species ID	*E. faecalis*
BST-B-3-2	Laughing Gull	Malaquite Beach	5/22/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-4-1	Laughing Gull	Malaquite Beach	5/22/2003	7/20/2003	Species ID	*E. faecalis*
BST-B-4-2	Laughing Gull	Malaquite Beach	5/22/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-4-3	Laughing Gull	Malaquite Beach	5/22/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-7-1	Laughing Gull	Malaquite Beach	5/22/2003	7/21/2003	Species ID	*E. faecalis*
BST-B-12-1	Laughing Gull	Rockport	6/2/2003	7/21/2003	Species ID	*E. faecalis*
BST-B-12-2	Laughing Gull	Rockport	6/2/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-12-3	Laughing Gull	Rockport	6/2/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-14-1	Laughing Gull	Rockport	6/2/2003	7/20/2003	Species ID	*E. faecalis*
BST-B-14-2	Laughing Gull	Rockport	6/2/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-14-3	Laughing Gull	Rockport	6/2/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-16-1	Laughing Gull	Rockport	6/2/2003	7/20/2003	Species ID	*E. faecalis*
BST-B-16-2	Laughing Gull	Rockport	6/2/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-16-3	Laughing Gull	Rockport	6/2/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-18-1	Laughing Gull	Rockport	6/2/2003	7/20/2003	Species ID	*E. faecalis*
BST-B-18-2	Laughing Gull	Rockport	6/2/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-18-3	Laughing Gull	Rockport	6/2/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-20-1	Laughing Gull	Rockport	6/2/2003	7/20/2003	Species ID	*E. faecalis*
BST-B-20-3	Laughing Gull	Rockport	6/2/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-23-3	Laughing Gull	Port Aransas	6/9/2003	7/26/2003	Species ID	*E. mundtii*
BST-B-24-1	Laughing Gull	Port Aransas	6/9/2003	7/20/2003	Species ID	*E. mundtii*
BST-B-24-2	Laughing Gull	Port Aransas	6/9/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-24-3	Laughing Gull	Port Aransas	6/9/2003	7/26/2003	Species ID	*E. mundtii*
BST-B-26-1	Laughing Gull	Port Aransas	6/9/2003	7/20/2003	Species ID	*E. faecium*
BST-B-26-2	Laughing Gull	Port Aransas	6/9/2003	7/24/2003	Species ID	*E. faecium*
BST-B-26-3	Laughing Gull	Port Aransas	6/9/2003	7/26/2003	Species ID	*E. faecium*
BST-B-29-1	Laughing Gull	Port Aransas	6/9/2003	7/20/2003	Species ID	*E. faecalis*
BST-B-29-2	Laughing Gull	Port Aransas	6/9/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-32-1	Laughing Gull	North Beach	6/16/2003	7/20/2003	Species ID	*E. faecalis*
BST-B-32-2	Laughing Gull	North Beach	6/16/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-32-3	Laughing Gull	North Beach	6/16/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-34-1	Laughing Gull	Cole Park	6/16/2003	7/20/2003	Species ID	*E. faecalis*
BST-B-34-2	Laughing Gull	Cole Park	6/16/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-34-3	Laughing Gull	Cole Park	6/16/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-37-1	Laughing Gull	Indian Point	6/23/2003	7/20/2003	Species ID	*E. faecalis*
BST-B-37-2	Laughing Gull	Indian Point	6/23/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-37-3	Laughing Gull	Indian Point	6/23/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-39-1	Laughing Gull	Port Aransas	7/9/2003	7/20/2003	Species ID	*E. faecalis*
BST-B-39-2	Laughing Gull	Port Aransas	7/9/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-39-3	Laughing Gull	Port Aransas	7/9/2003	7/26/2003	Species ID	*E. faecalis*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
BST-B-40-1	Brown Pelican	Port Aransas	7/9/2003	7/20/2003	Species ID	*E. mundtii*
BST-B-40-2	Brown Pelican	Port Aransas	7/9/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-40-3	Brown Pelican	Port Aransas	7/9/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-42-1	Duck	Port Aransas	7/9/2003	7/20/2003	Species ID	*E. mundtii*
BST-B-42-2	Duck	Port Aransas	7/9/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-42-3	Duck	Port Aransas	7/9/2003	7/26/2003	Species ID	*E. flavescens*
BST-B-43-1	Roseate Spoonbill	Port Aransas	7/9/2003	7/20/2003	Species ID	*E. faecalis*
BST-B-43-2	Roseate Spoonbill	Port Aransas	7/9/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-43-3	Roseate Spoonbill	Port Aransas	7/9/2003	7/26/2003	Species ID	*E. mundtii*
BST-B-44-1	Owl	Port Aransas	7/9/2003	7/20/2003	Species ID	*E. mundtii*
BST-B-44-2	Owl	Port Aransas	7/9/2003	7/24/2003	Species ID	*E. mundtii*
BST-B-44-3	Owl	Port Aransas	7/9/2003	7/26/2003	Species ID	*E. mundtii*
BST-B-45-1	Parakeet	Ocean Dr. CC	7/9/2003	7/21/2003	Species ID	*E. faecalis*
BST-B-45-2	Parakeet	Ocean Dr. CC	7/9/2003	7/24/2003	Species ID	*E. faecalis*
BST-B-45-3	Parakeet	Ocean Dr. CC	7/9/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-46-1	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-49-3	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-50-2	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-52-1	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-52-2	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-52-3	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-53-1	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. gallinarum*
BST-B-53-2	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. dispar*
BST-B-53-3	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. gallinarum*
BST-B-54-1	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-54-2	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-54-3	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-61-1	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-61-2	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-61-3	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-62-1	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. raffinosus*
BST-B-62-2	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-62-3	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-63-1	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-63-2	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-63-3	Laughing Gull	Port Aransas	7/21/2003	7/26/2003	Species ID	*E. faecalis*
BST-B-66-3	Laughing Gull	Port Aransas	7/21/2003	7/30/2003	Species ID	*E. faecalis*
BST-B-67-1	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. faecalis*
BST-B-67-2	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. faecalis*
BST-B-67-3	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. faecalis*
BST-B-69-1	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. faecalis*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
BST-B-69-2	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. faecalis*
BST-B-70-1	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. faecalis*
BST-B-70-2	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. faecalis*
BST-B-70-3	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. faecalis*
BST-B-71-1	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. faecalis*
BST-B-73-1	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. faecalis*
BST-B-73-2	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. faecalis*
BST-B-73-3	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. faecalis*
BST-B-75-1	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. faecalis*
BST-B-75-2	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. casseliflavus*
BST-B-75-3	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. flavescens*
BST-B-76-1	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. faecalis*
BST-B-77-2	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. faecalis*
BST-B-77-3	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. faecalis*
BST-B-78-1	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. mundtii*
BST-B-78-2	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. faecalis*
BST-B-78-3	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. mundtii*
BST-B-79-1	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. faecalis*
BST-B-79-2	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. faecalis*
BST-B-80-1	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. faecalis*
BST-B-80-2	Laughing Gull	Seawall C.C.	7/22/2003	7/28/2003	Species ID	*E. faecalis*
BST-B-81-2	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. faecalis*
BST-B-82-2	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. faecalis*
BST-B-83-1	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. hirae*
BST-B-83-2	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. gallinarum*
BST-B-84-1	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. faecalis*
BST-B-84-2	Laughing Gull	Seawall C.C.	7/22/2003	7/30/2003	Species ID	*E. faecalis*
BST-C-1-1	Bovine	Rockport	6/2/2003	7/18/2003	Species ID	*E. gallinarum*
BST-C-1-2	Bovine	Rockport	6/2/2003	7/24/2003	Species ID	*E. mundtii*
BST-C-3-1	Bovine	Rockport	6/2/2003	7/18/2003	Species ID	*E. gallinarum*
BST-C-3-2	Bovine	Rockport	6/2/2003	7/24/2003	Species ID	*E. flavescens*
BST-C-6-1	Bovine	Rockport	6/2/2003	7/18/2003	Species ID	*E. casseliflavus*
BST-C-6-2	Bovine	Rockport	6/2/2003	7/22/2003	Species ID	*E. gallinarum*
BST-C-6-3	Bovine	Rockport	6/2/2003	7/22/2003	Species ID	*E. flavescens*
BST-C-7-1	Bovine	Rockport	6/2/2003	7/18/2003	Species ID	*E. flavescens*
BST-C-7-2	Bovine	Rockport	6/2/2003	7/24/2003	Species ID	*E. flavescens*
BST-C-7-3	Bovine	Rockport	6/2/2003	7/24/2003	Species ID	*E. flavescens*
BST-C-9-1	Bovine	Rockport	6/2/2003	7/18/2003	Species ID	*E. casseliflavus*
BST-C-9-2	Bovine	Rockport	6/2/2003	7/24/2003	Species ID	*E. casseliflavus*
BST-C-9-3	Bovine	Rockport	6/2/2003	7/24/2003	Species ID	*E. casseliflavus*
BST-C-11-1	Bovine	Rockport	6/2/2003	7/18/2003	Species ID	*E. casseliflavus*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
BST-C-11-2	Bovine	Rockport	6/2/2003	7/22/2003	Species ID	*E. casseliflavus*
BST-C-14-1	Bovine	Rockport	6/2/2003	7/18/2003	Species ID	*E. mundtii*
BST-C-14-2	Bovine	Rockport	6/2/2003	7/24/2003	Species ID	*E. mundtii*
BST-C-14-3	Bovine	Rockport	6/2/2003	7/24/2003	Species ID	*E. sulfureus*
BST-C-17-3	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. mundtii*
BST-C-18-2	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. flavescens*
BST-C-19-1	Bovine	Annaville	6/17/2003	7/18/2003	Species ID	*E. casseliflavus*
BST-C-22-3	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. mundtii*
BST-C-23-3	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. flavescens*
BST-C-24-2	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. mundtii*
BST-C-24-3	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. mundtii*
BST-C-25-1	Bovine	Annaville	6/17/2003	7/18/2003	Species ID	*E. mundtii*
BST-C-25-3	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. mundtii*
BST-C-26-2	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. mundtii*
BST-C-26-3	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. mundtii*
BST-C-27-1	Bovine	Annaville	6/17/2003	7/18/2003	Species ID	*E. mundtii*
BST-C-27-2	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. mundtii*
BST-C-27-3	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. mundtii*
BST-C-28-2	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. gallinarum*
BST-C-29-1	Bovine	Annaville	6/17/2003	7/18/2003	Species ID	*E. mundtii*
BST-C-29-2	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. mundtii*
BST-C-29-3	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. mundtii*
BST-C-30-2	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. mundtii*
BST-C-30-3	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. mundtii*
BST-C-31-1	Bovine	Annaville	6/17/2003	7/18/2003	Species ID	*E. mundtii*
BST-C-31-2	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. mundtii*
BST-C-32-1	Bovine	Annaville	6/17/2003	7/18/2003	Species ID	*E. mundtii*
BST-C-32-3	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. mundtii*
BST-C-34-1	Bovine	Annaville	6/17/2003	7/18/2003	Species ID	*E. mundtii*
BST-C-34-3	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. flavescens*
BST-C-35-2	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. casseliflavus*
BST-C-35-3	Bovine	Annaville	6/17/2003	7/23/2003	Species ID	*E. casseliflavus*
BST-C-36-1	Bovine	Annaville	6/17/2003	7/18/2003	Species ID	*E. mundtii*
BST-C-36-2	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. mundtii*
BST-C-36-3	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. mundtii*
BST-C-37-2	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. mundtii*
BST-C-37-3	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. mundtii*
BST-C-38-1	Bovine	Annaville	6/17/2003	7/18/2003	Species ID	*E. mundtii*
BST-C-38-2	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. mundtii*
BST-C-38-3	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. mundtii*
BST-C-39-1	Bovine	Annaville	6/17/2003	7/18/2003	Species ID	*E. mundtii*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
BST-C-39-2	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. gallinarum*
BST-C-39-3	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. mundtii*
BST-C-40-2	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. flavescens*
BST-C-40-3	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. flavescens*
BST-C-41-2	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. mundtii*
BST-C-41-3	Bovine	Annaville	6/17/2003	7/22/2003	Species ID	*E. mundtii*
BST-C-42-1	Bovine	Annaville	6/17/2003	7/18/2003	Species ID	*E. mundtii*
BST-C-42-2	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. faecalis*
BST-C-42-3	Bovine	Annaville	6/17/2003	7/24/2003	Species ID	*E. gallinarum*
BST-C-43-1	Bovine	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. faecalis*
BST-C-43-2	Bovine	Saratoga/Greenwood	6/26/2003	7/24/2003	Species ID	*E. faecalis*
BST-C-43-3	Bovine	Saratoga/Greenwood	6/26/2003	7/25/2003	Species ID	*E. faecalis**
BST-C-44-1	Bovine	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. gallinarum*
BST-C-44-2	Bovine	Saratoga/Greenwood	6/26/2003	7/22/2003	Species ID	*E. casseliflavus*
BST-C-44-3	Bovine	Saratoga/Greenwood	6/26/2003	7/22/2003	Species ID	*E. casseliflavus*
BST-C-45-1	Bovine	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. faecalis*
BST-C-45-2	Bovine	Saratoga/Greenwood	6/26/2003	7/24/2003	Species ID	*E. faecalis*
BST-C-45-3	Bovine	Saratoga/Greenwood	6/26/2003	7/24/2003	Species ID	*E. faecalis*
BST-C-46-2	Bovine	Saratoga/Greenwood	6/26/2003	7/24/2003	Species ID	*E. faecalis*
BST-C-46-3	Bovine	Saratoga/Greenwood	6/26/2003	7/25/2003	Species ID	*E. faecalis*
BST-C-47-1	Bovine	Saratoga/Greenwood	6/26/2003	N/A	Species ID	*E. casseliflavus*
BST-C-47-2	Bovine	Saratoga/Greenwood	6/26/2003	7/24/2003	Species ID	*E. casseliflavus*
BST-C-47-3	Bovine	Saratoga/Greenwood	6/26/2003	7/24/2003	Species ID	*E. casseliflavus*
BST-C-49-2	Bovine	Rockport	6/30/2003	7/24/2003	Species ID	*E. mundtii*
BST-C-50-2	Bovine	Rockport	6/30/2003	7/22/2003	Species ID	*E. faecalis*
BST-C-50-3	Bovine	Rockport	6/30/2003	7/22/2003	Species ID	*E. flavescens*
BST-C-52-1	Bovine	Rockport	6/30/2003	7/18/2003	Species ID	*E. casseliflavus*
BST-C-52-2	Bovine	Rockport	6/30/2003	7/22/2003	Species ID	*E. flavescens*
BST-C-52-3	Bovine	Rockport	6/30/2003	7/22/2003	Species ID	*E. casseliflavus*
BST-C-53-1	Bovine	Rockport	6/30/2003	7/30/2003	Species ID	*E. casseliflavus*
BST-C-54-1	Bovine	Rockport	6/30/2003	7/30/2003	Species ID	*E. faecalis*
BST-C-55-1	Bovine	Rockport	6/30/2003	7/30/2003	Species ID	*E. faecalis*
BST-C-55-2	Bovine	Rockport	6/30/2003	7/30/2003	Species ID	*E. casseliflavus*
BST-D-1-1	Black Lab	Alameda/Everhart	5/28/2003	7/18/2003	Species ID	*E. hirae*
BST-D-1-2	Black Lab	Alameda/Everhart	5/28/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-1-3	Black Lab	Alameda/Everhart	5/28/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-2-1	Yellow Lab	Alameda/Everhart	5/28/2003	7/18/2003	Species ID	*E. mundtii*
BST-D-2-3	Yellow Lab	Alameda/Everhart	5/28/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-3-1	Shih Tzu	Alameda/Everhart	5/28/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-3-2	Shih Tzu	Alameda/Everhart	5/28/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-3-3	Shih Tzu	Alameda/Everhart	5/28/2003	7/28/2003	Species ID	*E. mundtii*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
BST-D-4-1	Canine	Alameda/Everhart	5/28/2003	7/18/2003	Species ID	*E. mundtii*
BST-D-4-2	Canine	Alameda/Everhart	5/28/2003	7/22/2003	Species ID	*E. mundtii*
BST-D-4-3	Canine	Alameda/Everhart	5/28/2003	7/22/2003	Species ID	*E. mundtii*
BST-D-5-2	Mix Canine	Malaquite Beach	5/29/2003	7/22/2003	Species ID	*E. faecalis*
BST-D-6-1	Canine	Bird Island Basin	5/29/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-6-2	Canine	Bird Island Basin	5/29/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-6-3	Canine	Bird Island Basin	5/29/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-7-3	Lab Mix	Rockport	6/2/2003	7/22/2003	Species ID	*E. gallinarum*
BST-D-8-1	Rottweiler Mix	Rockport	6/2/2003	7/18/2003	Species ID	*E. mundtii*
BST-D-8-2	Rottweiler Mix	Rockport	6/2/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-8-3	Rottweiler Mix	Rockport	6/2/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-9-1	Staffordshire Terrier	Yorktown	6/2/2003	7/18/2003	Species ID	*E. hirae*
BST-D-9-2	Staffordshire Terrier	Yorktown	6/2/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-9-3	Staffordshire Terrier	Yorktown	6/2/2003	7/28/2003	Species ID	*E. gallinarum*
BST-D-10-1	Rottweiler	Yorktown	6/2/2003	7/18/2003	Species ID	*E. mundtii*
BST-D-10-3	Rottweiler	Yorktown	6/2/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-11-2	German Shepard	Yorktown	6/2/2003	7/22/2003	Species ID	*E. mundtii*
BST-D-11-3	German Shepard	Yorktown	6/2/2003	7/22/2003	Species ID	*E. mundtii*
BST-D-12-2	Staffordshire Terrier	Yorktown	6/2/2003	7/22/2003	Species ID	*E. faecium*
BST-D-12-3	Staffordshire Terrier	Yorktown	6/2/2003	7/22/2003	Species ID	*E. gallinarum*
BST-D-16-1	Miniature Poodle	Yorktown	6/2/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-18-1	Long-Haired Dachshund	Wooldridge	6/9/2003	7/18/2003	Species ID	*E. gallinarum*
BST-D-18-2	Long-Haired Dachshund	Wooldridge	6/9/2003	7/28/2003	Species ID	*E. faecium*
BST-D-18-3	Long-Haired Dachshund	Wooldridge	6/9/2003	7/28/2003	Species ID	*E. gallinarum*
BST-D-19-1	Jack Russel Terrier	Wooldridge	6/9/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-19-2	Jack Russel Terrier	Wooldridge	6/9/2003	7/28/2003	Species ID	*E. casseliflavus*
BST-D-19-3	Jack Russel Terrier	Wooldridge	6/9/2003	7/28/2003	Species ID	*E. casseliflavus*
BST-D-20-1	Toy Poodle	Wooldridge	6/9/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-20-2	Toy Poodle	Wooldridge	6/9/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-20-3	Toy Poodle	Wooldridge	6/9/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-21-1	Mix Canine	Wooldridge	6/9/2003	7/18/2003	Species ID	*E. mundtii*
BST-D-21-2	Mix Canine	Wooldridge	6/9/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-21-3	Mix Canine	Wooldridge	6/9/2003	7/28/2003	Species ID	*E. gallinarum*
BST-D-22-1	Beagle	Wooldridge	6/9/2003	7/18/2003	Species ID	*E. faecium*
BST-D-22-2	Beagle	Wooldridge	6/9/2003	7/28/2003	Species ID	*E. faecium*
BST-D-22-3	Beagle	Wooldridge	6/9/2003	7/28/2003	Species ID	*E. faecium*
BST-D-23-2	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-24-1	Jack Russel Terrier	Saratoga/Cabaniss	6/23/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-24-2	Jack Russel Terrier	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-24-3	Jack Russel Terrier	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-25-1	Shepard Mix	Saratoga/Cabaniss	6/23/2003	7/18/2003	Species ID	*E. faecalis*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
BST-D-25-2	Shepard Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-25-3	Shepard Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-26-1	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-26-2	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-26-3	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-27-1	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-27-2	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-27-3	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. faecium*
BST-D-28-2	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/22/2003	Species ID	*E. hirae*
BST-D-28-3	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/22/2003	Species ID	*E. mundtii*
BST-D-29-1	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/18/2003	Species ID	*E. faecium*
BST-D-29-2	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/22/2003	Species ID	*E. faecalis*
BST-D-29-3	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/23/2003	Species ID	*E. faecalis*
BST-D-30-1	Shepard Mix	Saratoga/Cabaniss	6/23/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-31-3	Pit Bull	Saratoga/Cabaniss	6/23/2003	7/22/2003	Species ID	*E. faecalis*
BST-D-32-1	Pit Bull	Saratoga/Cabaniss	6/23/2003	7/18/2003	Species ID	*E. gallinarum*
BST-D-32-2	Pit Bull	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-33-1	Chow Mix	Saratoga/Cabaniss	6/23/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-33-2	Chow Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-33-3	Chow Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-34-1	Border Collie Mix	Saratoga/Cabaniss	6/23/2003	7/18/2003	Species ID	*E. mundtii*
BST-D-34-2	Border Collie Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-34-3	Border Collie Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. flavescens*
BST-D-35-1	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/18/2003	Species ID	*E. hirae*
BST-D-35-3	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/22/2003	Species ID	*E. faecium*
BST-D-36-1	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/18/2003	Species ID	*E. mundtii*
BST-D-36-2	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. dispar*
BST-D-36-3	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-37-1	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-37-2	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-37-3	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. hirae*
BST-D-38-1	Terrier	Saratoga/Cabaniss	6/23/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-38-2	Terrier	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. faecium*
BST-D-38-3	Terrier	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-39-1	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/18/2003	Species ID	*E. mundtii*
BST-D-39-3	Lab Mix	Saratoga/Cabaniss	6/23/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-40-1	Mix Canine	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-40-2	Mix Canine	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-40-3	Mix Canine	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-41-1	Mix Canine	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. faecium*
BST-D-41-2	Mix Canine	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. mundtii*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
BST-D-42-1	Chihuahua Mix	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-42-2	Chihuahua Mix	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-42-3	Chihuahua Mix	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-43-1	Spaniel Mix	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. flavescens*
BST-D-43-2	Spaniel Mix	Saratoga/Greenwood	6/26/2003	7/22/2003	Species ID	*E. flavescens*
BST-D-43-3	Spaniel Mix	Saratoga/Greenwood	6/26/2003	7/22/2003	Species ID	*E. casseliflavus*
BST-D-44-1	Rottweiler Mix	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-44-2	Rottweiler Mix	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-44-3	Rottweiler Mix	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-45-1	Mix Canine	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. hirae*
BST-D-46-1	Mix Canine	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. hirae*
BST-D-46-2	Mix Canine	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. mundtii*
BST-D-46-3	Mix Canine	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-47-1	Mix Canine	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. faecalis*
BST-D-47-2	Mix Canine	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. faecalis*
BST-D-48-1	Mix Canine	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. mundtii*
BST-D-48-2	Mix Canine	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. flavescens*
BST-D-48-3	Mix Canine	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. mundtii*
BST-D-49-1	Mix Canine	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. gallinarum*
BST-D-49-2	Mix Canine	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. hirae*
BST-D-49-3	Mix Canine	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. hirae*
BST-D-50-1	Canine	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. gallinarum*
BST-D-50-2	Canine	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. gallinarum*
BST-D-50-3	Canine	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. hirae*
BST-D-51-1	Canine	Saratoga/Greenwood	6/26/2003	7/18/2003	Species ID	*E. faecium*
BST-D-51-2	Canine	Saratoga/Greenwood	6/26/2003	7/28/2003	Species ID	*E. gallinarum*
BST-D-52-2	Canine	Saratoga/Greenwood	6/26/2003	7/22/2003	Species ID	*E. faecium*
BST-D-52-3	Canine	Saratoga/Greenwood	6/26/2003	7/22/2003	Species ID	*E. mundtii*
BST-D-53-2	Canine	Saratoga/Greenwood	6/26/2003	7/22/2003	Species ID	*E. mundtii*
BST-D-53-3	Canine	Saratoga/Greenwood	6/26/2003	7/23/2003	Species ID	*E. faecium*
BST-H-1-1	Human	J.P. Luby Beach	6/30/2003	7/20/2003	Species ID	*E. gallinarum*
BST-H-1-3	Human	J.P. Luby Beach	6/30/2003	7/26/2003	Species ID	*E. gallinarum*
BST-H-2-1	Human	J.P. Luby Beach	6/30/2003	7/20/2003	Species ID	*E. faecium*
BST-H-2-2	Human	J.P. Luby Beach	6/30/2003	7/24/2003	Species ID	*E. casseliflavus*
BST-H-3-1	Human	J.P. Luby Beach	6/30/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-3-2	Human	J.P. Luby Beach	6/30/2003	7/24/2003	Species ID	*E. faecalis*
BST-H-3-3	Human	J.P. Luby Beach	6/30/2003	7/27/2003	Species ID	*E. faecalis*
BST-H-4-1	Human	J.P. Luby Beach	6/30/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-4-2	Human	J.P. Luby Beach	6/30/2003	7/24/2003	Species ID	*E. faecalis*
BST-H-4-3	Human	J.P. Luby Beach	6/30/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-5-1	Human	J.P. Luby Beach	6/30/2003	7/20/2003	Species ID	*E. faecalis*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
BST-H-5-2	Human	J.P. Luby Beach	6/30/2003	7/24/2003	Species ID	*E. faecalis*
BST-H-5-3	Human	J.P. Luby Beach	6/30/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-6-1	Human	J.P. Luby Beach	6/30/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-6-2	Human	J.P. Luby Beach	6/30/2003	7/24/2003	Species ID	*E. faecalis*
BST-H-6-3	Human	J.P. Luby Beach	6/30/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-7-1	Human	J.P. Luby Beach	6/30/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-7-2	Human	J.P. Luby Beach	6/30/2003	7/24/2003	Species ID	*E. faecalis*
BST-H-7-3	Human	J.P. Luby Beach	6/30/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-8-1	Human	J.P. Luby Beach	6/30/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-10-1	Human	J.P. Luby Beach	6/30/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-10-3	Human	J.P. Luby Beach	6/30/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-11-1	Human	J.P. Luby Beach	6/30/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-11-2	Human	J.P. Luby Beach	6/30/2003	7/24/2003	Species ID	*E. faecalis*
BST-H-11-3	Human	J.P. Luby Beach	6/30/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-12-1	Human	J.P. Luby Beach	6/30/2003	7/20/2003	Species ID	*E. faecium*
BST-H-12-3	Human	J.P. Luby Beach	6/30/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-13-1	Human	J.P. Luby Beach	6/30/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-13-2	Human	J.P. Luby Beach	6/30/2003	7/24/2003	Species ID	*E. faecalis*
BST-H-13-3	Human	J.P. Luby Beach	6/30/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-14-3	Human	Port Aransas	6/30/2003	7/22/2003	Species ID	*E. faecalis*
BST-H-15-1	Human	Port Aransas	6/30/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-15-3	Human	Port Aransas	6/30/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-16-1	Human	Port Aransas	6/30/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-16-2	Human	Port Aransas	6/30/2003	7/24/2003	Species ID	*E. faecalis*
BST-H-16-3	Human	Port Aransas	6/30/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-17-1	Human	Mustang Island	6/30/2003	7/20/2003	Species ID	*E. avium*
BST-H-17-2	Human	Mustang Island	6/30/2003	7/24/2003	Species ID	*E. faecalis*
BST-H-17-3	Human	Mustang Island	6/30/2003	7/26/2003	Species ID	*E. avium*
BST-H-18-1	Human	Mustang Island	6/30/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-18-2	Human	Mustang Island	6/30/2003	7/24/2003	Species ID	*E. faecalis*
BST-H-18-3	Human	Mustang Island	6/30/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-19-1	Human	Mustang Island	6/30/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-19-2	Human	Mustang Island	6/30/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-19-3	Human	Mustang Island	6/30/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-20-1	Human	Mustang Island	6/30/2003	7/20/2003	Species ID	*E. flavescens*
BST-H-20-2	Human	Mustang Island	6/30/2003	7/26/2003	Species ID	*E. flavescens*
BST-H-20-3	Human	Mustang Island	6/30/2003	7/27/2003	Species ID	*E. flavescens*
BST-H-21-1	Human	Mustang Island	6/30/2003	7/20/2003	Species ID	*E. flavescens*
BST-H-21-2	Human	Mustang Island	6/30/2003	7/26/2003	Species ID	*E. flavescens*
BST-H-21-3	Human	Mustang Island	6/30/2003	7/26/2003	Species ID	*E. gallinarum*
BST-H-22-1	Human	Mustang Island	6/30/2003	7/20/2003	Species ID	*E. flavescens*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
BST-H-23-1	Human	Padre Island	6/30/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-23-2	Human	Padre Island	6/30/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-23-3	Human	Padre Island	6/30/2003	7/26/2003	Species ID	*E. gallinarum*
BST-H-25-1	Human	Padre Island	6/30/2003	7/20/2003	Species ID	*E. gallinarum*
BST-H-25-2	Human	Padre Island	6/30/2003	7/26/2003	Species ID	*E. gallinarum*
BST-H-25-3	Human	Padre Island	6/30/2003	7/26/2003	Species ID	*E. gallinarum*
BST-H-26-1	Human	Padre Island	6/30/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-26-2	Human	Padre Island	6/30/2003	7/26/2003	Species ID	*E. casseliflavus*
BST-H-27-1	Human	Ocean	7/9/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-27-3	Human	Ocean	7/9/2003	7/26/2003	Species ID	*E. gallinarum*
BST-H-28-1	Human	Ocean	7/8/2003	7/20/2003	Species ID	*E. gallinarum*
BST-H-28-2	Human	Ocean	7/8/2003	7/26/2003	Species ID	*E. gallinarum*
BST-H-28-3	Human	Ocean	7/8/2003	7/26/2003	Species ID	*E. gallinarum*
BST-H-30-1	Human	Mustang Island	7/9/2003	7/20/2003	Species ID	*E. casseliflavus*
BST-H-30-2	Human	Mustang Island	7/9/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-30-3	Human	Mustang Island	7/9/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-31-2	Human	Mustang Island	7/9/2003	7/22/2003	Species ID	*E. flavescens*
BST-H-31-3	Human	Mustang Island	7/9/2003	7/24/2003	Species ID	*E. faecalis*
BST-H-33-1	Human	J.P. Luby Beach	7/9/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-33-2	Human	J.P. Luby Beach	7/9/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-33-3	Human	J.P. Luby Beach	7/9/2003	7/26/2003	Species ID	*E. faecalis*
BST-H-34-1	Human	J.P. Luby Beach	7/9/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-34-2	Human	J.P. Luby Beach	7/9/2003	7/27/2003	Species ID	*E. faecium*
BST-H-34-3	Human	J.P. Luby Beach	7/9/2003	7/26/2003	Species ID	*E. faecium*
BST-H-36-1	Human	J.P. Luby Beach	7/9/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-36-2	Human	J.P. Luby Beach	7/9/2003	7/26/2003	Species ID	*E. gallinarum*
BST-H-37-1	Human	J.P. Luby Beach	7/9/2003	7/20/2003	Species ID	*E. gallinarum*
BST-H-37-2	Human	J.P. Luby Beach	7/9/2003	7/26/2003	Species ID	*E. gallinarum*
BST-H-37-3	Human	J.P. Luby Beach	7/9/2003	7/26/2003	Species ID	*E. casseliflavus*
BST-H-38-1	Human	J.P. Luby Beach	7/9/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-38-3	Human	J.P. Luby Beach	7/9/2003	7/26/2003	Species ID	*E. casseliflavus*
BST-H-39-1	Human	J.P. Luby Beach	7/9/2003	7/20/2003	Species ID	*E. casseliflavus*
BST-H-39-3	Human	J.P. Luby Beach	7/9/2003	7/28/2003	Species ID	*E. faecalis*
BST-H-41-2	Human	J.P. Luby Beach	7/9/2003	7/22/2003	Species ID	*E. faecalis*
BST-H-42-2	Human	J.P. Luby Beach	7/9/2003	7/22/2003	Species ID	*E. casseliflavus*
BST-H-42-3	Human	J.P. Luby Beach	7/9/2003	7/22/2003	Species ID	*E. flavescens*
BST-H-44-1	Human	S. Texas/Ocean	7/9/2003	7/20/2003	Species ID	*E. gallinarum*
BST-H-44-2	Human	S. Texas/Ocean	7/9/2003	7/28/2003	Species ID	*E. dispar*
BST-H-46-1	Human	Ocean	7/10/2003	7/20/2003	Species ID	*E. faecalis*
BST-H-46-2	Human	Ocean	7/10/2003	7/28/2003	Species ID	*E. faecalis*
BST-H-46-3	Human	Ocean	7/10/2003	7/28/2003	Species ID	*E. faecalis*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
BST-H-48-2	Human	Rockport	7/12/2003	7/22/2003	Species ID	*E. faecium*
BST-H-48-3	Human	Rockport	7/12/2003	7/22/2003	Species ID	*E. faecium*
BST-H-50-1	Human	Rockport	7/12/2003	7/22/2003	Species ID	*E. faecalis*
BST-H-50-2	Human	Rockport	7/12/2003	7/22/2003	Species ID	*E. faecalis*
BST-H-50-3	Human	Rockport	7/12/2003	7/23/2003	Species ID	*E. faecalis*
BST-H-53-1	Human	Indiana/Santa Fe	7/17/2003	7/24/2003	Species ID	*E. faecalis*
BST-H-53-2	Human	Indiana/Santa Fe	7/17/2003	7/25/2003	Species ID	*E. faecalis*
BST-H-53-3	Human	Indiana/Santa Fe	7/17/2003	7/24/2003	Species ID	*E. faecalis*
BST-H-55-1	Human	Ocean	7/22/2003	7/30/2003	Species ID	*E. faecalis*
BST-H-55-2	Human	Ocean	7/22/2003	7/30/2003	Species ID	*E. faecalis*
BST-H-56-2	Human	Ocean	7/26/2003	7/30/2003	Species ID	*E. faecalis*
OST-B-100-1	White Crane	2642 Hwy 763 Barren Field	12/11/2009	7/14/2010	Species ID	*E. faecalis*
OST-B-102-1	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. hirae*
OST-B-102-10	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecium*
OST-B-102-11	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecium*
OST-B-102-2	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-102-4	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. mundtii*
OST-B-102-6	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecium*
OST-B-102-7	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecium*
OST-B-103-1	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecium*
OST-B-103-2	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecium*
OST-B-103-3	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-103-4	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecium*
OST-B-103-5	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecium*
OST-B-103-6	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecium*
OST-B-103-7	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-104-1	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Genus ID	*E. faecium*
OST-B-104-10	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. mundtii*
OST-B-104-11	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecium*
OST-B-104-12	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Genus ID	*E. hirae*
OST-B-104-2	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecium*
OST-B-104-4	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Genus ID	*E. faecium*
OST-B-104-5	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Genus ID	*E. faecium*
OST-B-104-7	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	9/29/2010	Species ID	*E. faecium*
OST-B-105-1	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	8/6/2010	Genus ID	*E. mundtii*
OST-B-105-10	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	8/6/2010	Genus ID	*E. hirae*
OST-B-105-11	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. mundtii*
OST-B-105-2	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. mundtii*
OST-B-105-3	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. hirae*
OST-B-105-4	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. mundtii*
OST-B-105-5	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. durans*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-B-105-6	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. mundtii*
OST-B-105-7	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. hirae*
OST-B-105-8	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. mundtii*
OST-B-105-9	Domestic Chicken	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. mundtii*
OST-B-106-1	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-106-2	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-106-3	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-106-4	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-106-5	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-106-6	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-106-7	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-106-8	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-107-1	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-107-2	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-107-3	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-107-4	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-107-5	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-107-6	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-107-7	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-107-8	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-108-1	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-108-10	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-108-11	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. gallinarum*
OST-B-108-12	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Genus ID	*E. casseliflavus*
OST-B-108-2	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. flavescens*
OST-B-108-3	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-108-4	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Genus ID	*E. casseliflavus*
OST-B-108-5	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-108-6	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. casseliflavus*
OST-B-108-7	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. casseliflavus*
OST-B-108-8	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. casseliflavus*
OST-B-108-9	Sparrow/Blackbird	Eileen Rogers Pasture	4/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-109-1	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-109-10	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Genus ID	*E. gallinarum*
OST-B-109-11	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. gallinarum*
OST-B-109-12	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. gallinarum*
OST-B-109-13	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. casseliflavus*
OST-B-109-14	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Genus ID	*E. gallinarum*
OST-B-109-15	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Genus ID	*E. flavescens*
OST-B-109-16	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-109-2	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. faecalis*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-B-109-3	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. casseliflavus*
OST-B-109-4	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. flavescens*
OST-B-109-5	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. gallinarum*
OST-B-109-7	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. casseliflavus*
OST-B-109-8	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. casseliflavus*
OST-B-109-9	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. casseliflavus*
OST-B-110-1	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-110-10	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-110-11	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-110-12	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. mundtii*
OST-B-110-13	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-110-14	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-110-15	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-110-16	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-110-2	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-110-3	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-110-4	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-110-5	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-110-6	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-110-7	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Species ID	*E. faecalis*
OST-B-110-9	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/29/2010	Genus ID	*E. mundtii*
OST-B-111-1	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-111-10	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-111-11	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-111-12	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-111-13	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Genus ID	*E. mundtii*
OST-B-111-14	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-111-15	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Genus ID	*E. faecium*
OST-B-111-16	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-111-3	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-111-4	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-111-5	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-111-6	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-111-7	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-111-8	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-111-9	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-112-1	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-112-10	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Genus ID	*E. faecium*
OST-B-112-11	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-112-13	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Genus ID	*E. faecium*
OST-B-112-14	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. mundtii*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-B-112-2	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-112-2	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-112-3	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. durans*
OST-B-112-4	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Genus ID	*E. mundtii*
OST-B-112-5	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. mundtii*
OST-B-112-6	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Genus ID	*E. durans*
OST-B-112-7	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. mundtii*
OST-B-112-8	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-113-1	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. gallinarum*
OST-B-113-10	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-113-12	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-113-13	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	10/7/2010	Genus ID	*E. faecalis*
OST-B-113-14	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-113-16	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-113-2	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-113-3	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-113-4	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-113-5	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-113-6	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-113-7	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-B-113-8	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-113-9	Grackle - Unknown	Fireworks Stand Agrilife	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-114-1	Cardinal	Agrilife Fields	8/24/2010	10/29/2010	Genus ID	*E. hirae*
OST-B-114-4	Cardinal	Agrilife Fields	8/24/2010	10/29/2010	Species ID	*E. durans*
OST-B-115-1	Cardinal	Agrilife Fields	8/24/2010	10/29/2010	Genus ID	*E. hirae*
OST-B-115-2	Cardinal	Agrilife Fields	8/24/2010	10/29/2010	Genus ID	*E. raffinosus*
OST-B-115-4	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecium*
OST-B-117-1	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-117-10	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-117-11	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-117-12	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-117-13	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-117-14	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-117-15	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-117-16	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-117-2	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-117-3	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-117-4	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-117-5	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-117-6	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-117-7	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-B-117-8	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-117-9	Cardinal	Agrilife Fields	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-B-121-1	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-121-10	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-121-11	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-121-12	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-B-121-13	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-121-14	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-121-15	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-121-16	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. faecium*
OST-B-121-17	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. faecium*
OST-B-121-2	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-121-20	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-B-121-6	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-121-7	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-121-8	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-B-121-9	Hering	Under Bridge - TCEQ GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-126-1	Duck	Yorktown Bridge - Yorktown Blvd	9/9/2010	11/10/2010	Species ID	*E. flavescens*
OST-B-126-10	Duck	Yorktown Bridge - Yorktown Blvd	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-126-12	Duck	Yorktown Bridge - Yorktown Blvd	9/9/2010	11/10/2010	Species ID	*E. gallinarum*
OST-B-126-13	Duck	Yorktown Bridge - Yorktown Blvd	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-126-14	Duck	Yorktown Bridge - Yorktown Blvd	9/9/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-126-17	Duck	Yorktown Bridge - Yorktown Blvd	9/9/2010	11/10/2010	Species ID	*E. casseliflavus*
OST-B-126-2	Duck	Yorktown Bridge - Yorktown Blvd	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-126-3	Duck	Yorktown Bridge - Yorktown Blvd	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-126-4	Duck	Yorktown Bridge - Yorktown Blvd	9/9/2010	11/10/2010	Species ID	*E. gallinarum*
OST-B-126-5	Duck	Yorktown Bridge - Yorktown Blvd	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-126-6	Duck	Yorktown Bridge - Yorktown Blvd	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-126-7	Duck	Yorktown Bridge - Yorktown Blvd	9/9/2010	11/10/2010	Species ID	*E. faecium*
OST-B-126-8	Duck	Yorktown Bridge - Yorktown Blvd	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-B-126-9	Duck	Yorktown Bridge - Yorktown Blvd	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-129-1	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. hirae*
OST-B-129-10	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-B-129-11	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-B-129-12	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-B-129-13	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-B-129-14	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecium*
OST-B-129-15	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-B-129-2	Songbird - Unknown	Agrilife Fields	9/9/2010	10/29/2010	Species ID	*E. casseliflavus*
OST-B-129-3	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. hirae*
OST-B-129-4	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. mundtii*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-B-129-5	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-B-129-6	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-B-129-7	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-B-129-8	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-B-129-9	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-B-132-1	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-132-10	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-132-11	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-132-12	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-132-13	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-132-14	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-132-15	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-132-16	Songbird - Unknown	Agrilife Fields	9/9/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-132-2	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-132-3	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-132-4	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-132-5	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-132-6	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-132-7	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-132-8	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-132-9	Songbird - Unknown	Agrilife Fields	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-133-1	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Genus ID	*E. faecalis*
OST-B-133-10	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecium*
OST-B-133-12	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecium*
OST-B-133-13	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecium*
OST-B-133-14	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecium*
OST-B-133-15	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. gallinarum*
OST-B-133-16	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecium*
OST-B-133-17	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Genus ID	*E. faecium*
OST-B-133-18	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. gallinarum*
OST-B-133-19	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecium*
OST-B-133-2	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-133-20	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. casseliflavus*
OST-B-133-3	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. casseliflavus*
OST-B-133-4	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecium*
OST-B-133-5	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecium*
OST-B-133-6	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecium*
OST-B-133-8	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-133-9	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. flavescens*
OST-B-134-1	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-134-11	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-B-134-12	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-134-13	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecium*
OST-B-134-14	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Genus ID	*E. casseliflavus*
OST-B-134-15	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-134-17	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-134-2	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-134-21	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-134-22	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-134-23	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-134-24	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-134-3	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-134-4	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-134-5	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-134-7	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecium*
OST-B-134-8	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-134-9	Grackle	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-136-1	Cow Bird	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-136-10	Cow Bird	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-136-13	Cow Bird	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-136-14	Cow Bird	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-136-16	Cow Bird	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-136-17	Cow Bird	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-136-3	Cow Bird	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-136-7	Cow Bird	Agrilife Fields	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-B-137-1	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-10	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-11	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-12	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-13	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-14	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-15	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-16	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-17	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. casseliflavus*
OST-B-137-18	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecium*
OST-B-137-19	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-2	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-3	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-4	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-5	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-6	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-7	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-B-137-8	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-137-9	Unknown - Bird	Memorial Garden Cemetary - TCEQ 18500	9/13/2010	10/29/2010	Species ID	*E. faecalis*
OST-B-138-2	Unknown - Bird	Corpus Christi International Airport	12/20/2010	4/6/2011	Species ID	*E. mundtii*
OST-B-138-3	Unknown - Bird	Corpus Christi International Airport	12/20/2010	4/6/2011	Species ID	*E. mundtii*
OST-B-138-5	Unknown - Bird	Corpus Christi International Airport	12/20/2010	4/6/2011	Species ID	*E. mundtii*
OST-B-141-1	Unknown - Bird	Corpus Christi International Airport	12/20/2010	4/6/2011	Species ID	*E. gallinarum*
OST-B-142-1	Unknown - Bird	Corpus Christi International Airport	12/20/2010	4/6/2011	Species ID	*E. flavescens*
OST-B-143-1	Unknown - Bird	Corpus Christi International Airport	12/20/2010	4/6/2011	Species ID	*E. faecalis*
OST-B-143-2	Unknown - Bird	Corpus Christi International Airport	12/20/2010	4/6/2011	Species ID	*E. faecalis*
OST-B-143-3	Unknown - Bird	Corpus Christi International Airport	12/20/2010	4/6/2011	Species ID	*E. faecalis*
OST-B-143-4	Unknown - Bird	Corpus Christi International Airport	12/20/2010	4/6/2011	Species ID	*E. faecalis*
OST-B-143-6	Unknown - Bird	Corpus Christi International Airport	12/20/2010	4/6/2011	Species ID	*E. faecalis*
OST-B-144-1	Unknown - Bird	Corpus Christi International Airport	1/26/2011	3/29/2011	Species ID	*E. flavescens*
OST-B-144-2	Unknown - Bird	Corpus Christi International Airport	1/26/2011	3/29/2011	Species ID	*E. casseliflavus*
OST-B-144-3	Unknown - Bird	Corpus Christi International Airport	1/26/2011	3/29/2011	Species ID	*E. faecalis*
OST-B-144-4	Unknown - Bird	Corpus Christi International Airport	1/26/2011	3/29/2011	Species ID	*E. casseliflavus*
OST-B-144-5	Unknown - Bird	Corpus Christi International Airport	1/26/2011	3/29/2011	Species ID	*E. casseliflavus*
OST-C-100-1	Bovine	TCEQ Station 18501	10/27/2009	9/29/2010	Species ID	*E. faecalis*
OST-C-100-2	Bovine	TCEQ Station 18501	10/27/2009	9/29/2010	Genus ID	*E. raffinosus*
OST-C-100-3	Bovine	TCEQ Station 18501	10/27/2009	9/29/2010	Species ID	*E. faecalis*
OST-C-101-2	Bovine	TCEQ Station 18501	10/27/2009	9/29/2010	Species ID	*E. faecalis*
OST-C-101-3	Bovine	TCEQ Station 18501	10/27/2009	9/29/2010	Genus ID	*E. faecium*
OST-C-101-4	Bovine	TCEQ Station 18501	10/27/2009	9/29/2010	Genus ID	*E. faecalis*
OST-C-107-2	Bovine	TCEQ Station 18501	10/27/2009	9/29/2010	Species ID	*E. gallinarum*
OST-C-109-2	Bovine	TCEQ Station 18501	10/27/2009	9/29/2010	Species ID	*E. flavescens*
OST-C-109-3	Bovine	TCEQ Station 18501	10/27/2009	9/29/2010	Genus ID	*E. casseliflavus*
OST-C-110-1	Bovine	TCEQ Station 18501	10/27/2009	7/14/2010	Species ID	*E. faecalis*
OST-C-110-3	Bovine	TCEQ Station 18501	10/27/2009	7/14/2010	Species ID	*E. casseliflavus*
OST-C-114-1	Bovine	Eileen Rogers Pasture	4/14/2010	7/14/2010	Genus ID	*E. gallinarum*
OST-C-114-2	Bovine	Eileen Rogers Pasture	4/14/2010	7/14/2010	Species ID	*E. faecalis*
OST-C-114-4	Bovine	Eileen Rogers Pasture	4/14/2010	7/14/2010	Genus ID	*E. gallinarum*
OST-C-118-3	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. flavescens*
OST-C-118-4	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-119-2	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. faecalis*
OST-C-119-3	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. faecalis*
OST-C-119-4	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. faecalis*
OST-C-119-5	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. faecalis*
OST-C-119-6	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. faecalis*
OST-C-119-7	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. faecalis*
OST-C-119-8	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. faecalis*
OST-C-121-1	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-C-121-2	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-121-3	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-121-7	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-122-2	Bovine	Eileen Rogers Pasture	4/14/2010	7/14/2010	Species ID	*E. casseliflavus*
OST-C-122-7	Bovine	Eileen Rogers Pasture	4/14/2010	7/14/2010	Species ID	*E. casseliflavus*
OST-C-123-3	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. casseliflavus*
OST-C-123-4	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. casseliflavus*
OST-C-123-5	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. gallinarum*
OST-C-123-6	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. casseliflavus*
OST-C-124-1	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. casseliflavus*
OST-C-124-2	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-124-4	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. flavescens*
OST-C-124-5	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. casseliflavus*
OST-C-125-1	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. gallinarum*
OST-C-125-2	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. gallinarum*
OST-C-125-3	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. gallinarum*
OST-C-125-4	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-125-6	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-125-7	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. casseliflavus*
OST-C-125-8	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-125-9	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-125-12	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. casseliflavus*
OST-C-126-2	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. durans*
OST-C-126-3	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-126-4	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. faecium*
OST-C-126-5	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. mundtii*
OST-C-126-6	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-127-1	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-127-6	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. mundtii*
OST-C-128-1	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Genus ID	*E. flavescens*
OST-C-128-4	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. casseliflavus*
OST-C-130-1	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Genus ID	*E. casseliflavus*
OST-C-130-2	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. gallinarum*
OST-C-130-4	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. casseliflavus*
OST-C-130-6	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. flavescens*
OST-C-132-1	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Genus ID	*E. gallinarum*
OST-C-132-2	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Genus ID	*E. gallinarum*
OST-C-132-3	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Genus ID	*E. casseliflavus*
OST-C-132-4	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Genus ID	*E. gallinarum*
OST-C-132-5	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. casseliflavus*
OST-C-132-6	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. casseliflavus*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-C-132-7	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. casseliflavus*
OST-C-133-1	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. casseliflavus*
OST-C-133-2	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. casseliflavus*
OST-C-133-3	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. casseliflavus*
OST-C-133-6	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Genus ID	*E. gallinarum*
OST-C-133-7	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. casseliflavus*
OST-C-134-1	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. flavescens*
OST-C-134-2	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. casseliflavus*
OST-C-134-3	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. gallinarum*
OST-C-134-4	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. casseliflavus*
OST-C-134-5	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. casseliflavus*
OST-C-134-6	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. casseliflavus*
OST-C-134-7	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Genus ID	*E. casseliflavus*
OST-C-134-8	Bovine	Eileen Rogers Pasture	4/14/2010	5/7/2010	Species ID	*E. casseliflavus*
OST-C-135-1	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-135-2	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. gallinarum*
OST-C-135-3	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-135-4	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-135-6	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-135-7	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-135-8	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. casseliflavus*
OST-C-136-1	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. mundtii*
OST-C-136-2	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. mundtii*
OST-C-136-3	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. hirae*
OST-C-136-4	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. faecalis*
OST-C-136-5	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. gallinarum*
OST-C-137-1	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Genus ID	*E. dispar*
OST-C-137-2	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. faecalis*
OST-C-137-5	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. faecalis*
OST-C-137-6	Bovine	Eileen Rogers Pasture	4/14/2010	7/9/2010	Species ID	*E. faecalis*
OST-C-138-1	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.gallinarum*
OST-C-138-2	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.casseliflavus*
OST-C-138-3	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-138-6	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.casseliflavus*
OST-C-138-7	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-138-8	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-139-1	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-140-1	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.casseliflavus*
OST-C-140-2	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-140-3	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-140-5	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.casseliflavus*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-C-141-3	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.casseliflavus*
OST-C-141-4	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.casseliflavus*
OST-C-141-5	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.casseliflavus*
OST-C-141-6	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.casseliflavus*
OST-C-142-2	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-142-3	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Genus ID	*E.casseliflavus*
OST-C-142-4	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-142-5	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.casseliflavus*
OST-C-143-1	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Species ID	*E.flavescens*
OST-C-143-2	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Species ID	*E.faecium*
OST-C-143-3	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Species ID	*E.faecium*
OST-C-143-4	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Species ID	*E.faecium*
OST-C-143-5	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Species ID	*E.faecium*
OST-C-144-1	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Genus ID	*E.gallinarum*
OST-C-146-1	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Genus ID	*E.casseliflavus*
OST-C-146-4	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Genus ID	*E.casseliflavus*
OST-C-146-5	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Genus ID	*E.casseliflavus*
OST-C-146-6	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Genus ID	*E.casseliflavus*
OST-C-147-1	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Genus ID	*E.faecium*
OST-C-147-2	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Species ID	*E.mundtii*
OST-C-147-3	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Genus ID	*E.faecium*
OST-C-147-4	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Genus ID	*E.mundtii*
OST-C-147-5	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Species ID	*E.faecium*
OST-C-148-2	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Genus ID	*E.mundtii*
OST-C-148-3	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Genus ID	*E.mundtii*
OST-C-148-4	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Species ID	*E.mundtii*
OST-C-148-5	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Species ID	*E.mundtii*
OST-C-148-6	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Species ID	*E.mundtii*
OST-C-149-1	Bovine	TCEQ Station 18501	12/20/2010	3/31/2011	Species ID	*E.casseliflavus*
OST-C-149-1	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-149-2	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-149-3	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.casseliflavus*
OST-C-149-4	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Genus ID	*E.gallinarum*
OST-C-149-5	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-150-1	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.casseliflavus*
OST-C-150-2	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-150-3	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.casseliflavus*
OST-C-150-4	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-150-5	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Genus ID	*E.flavescens*
OST-C-151-1	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.gallinarum*
OST-C-151-2	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-C-151-3	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-151-4	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-151-5	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-152-1	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Genus ID	*E.flavescens*
OST-C-153-2	Bovine	TCEQ Station 18501	12/20/2010	4/6/2011	Species ID	*E.flavescens*
OST-C-154-1	Bovine	Havelkas Residence - 3727 Co Rd61	1/26/2011	3/29/2011	Species ID	*E.casseliflavus*
OST-C-154-2	Bovine	Havelkas Residence - 3727 Co Rd61	1/26/2011	3/29/2011	Species ID	*E.casseliflavus*
OST-C-154-3	Bovine	Havelkas Residence - 3727 Co Rd61	1/26/2011	3/29/2011	Species ID	*E.casseliflavus*
OST-C-154-4	Bovine	Havelkas Residence - 3727 Co Rd61	1/26/2011	3/29/2011	Species ID	*E.flavescens*
OST-C-154-5	Bovine	Havelkas Residence - 3727 Co Rd61	1/26/2011	3/29/2011	Species ID	*E.casseliflavus*
OST-H-100I-2	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. faecalis*
OST-H-100I-4	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. gallinarum*
OST-H-100I-5	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. faecalis*
OST-H-100I-6	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. faecalis*
OST-H-100I-10	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. faecalis*
OST-H-100I-11	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. malodoratus*
OST-H-100I-12	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. faecium*
OST-H-101I-1	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Genus ID	*E. faecium*
OST-H-101I-2	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Genus ID	*E. faecium*
OST-H-101I-5	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Genus ID	*E. faecium*
OST-H-101I-10	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. faecium*
OST-H-102I-2	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Genus ID	*E. mundtii*
OST-H-102I-3	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Genus ID	*E. mundtii*
OST-H-102I-4	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Genus ID	*E. faecium*
OST-H-102I-5	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Genus ID	*E. faecalis*
OST-H-102I-7	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Genus ID	*E. mundtii*
OST-H-102I-9	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. mundtii*
OST-H-102I-11	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. faecium*
OST-H-103I-4	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. faecalis*
OST-H-103I-6	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Genus ID	*E. mundtii*
OST-H-103I-8	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Genus ID	*E. faecium*
OST-H-104I-2	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. faecium*
OST-H-104I-4	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. gallinarum*
OST-H-104I-5	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. faecalis*
OST-H-104I-6	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. faecium*
OST-H-104I-7	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. mundtii*
OST-H-104I-8	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. faecalis*
OST-H-104I-10	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. faecium*
OST-H-104I-11	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. faecalis*
OST-H-110E-4	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Genus ID	*E. gallinarum*
OST-H-110E-9	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Genus ID	*E. gallinarum*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-H-110E-10	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Genus ID	*E. casseliflavus*
OST-H-110E-11	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. casseliflavus*
OST-H-110E-12	Sewage	Robstown WWTP	3/10/2010	8/4/2010	Species ID	*E. casseliflavus*
OST-H-111E-1	Sewage	Robstown WWTP	3/10/2010	8/6/2010	Species ID	*E. casseliflavus*
OST-H-111E-4	Sewage	Robstown WWTP	3/10/2010	8/6/2010	Genus ID	*E. casseliflavus*
OST-H-111E-5	Sewage	Robstown WWTP	3/10/2010	8/6/2010	Genus ID	*E. gallinarum*
OST-H-111E-6	Sewage	Robstown WWTP	3/10/2010	8/6/2010	Species ID	*E. flavescens*
OST-H-111E-7	Sewage	Robstown WWTP	3/10/2010	8/6/2010	Genus ID	*E. faecalis*
OST-H-111E-8	Sewage	Robstown WWTP	3/10/2010	8/6/2010	Species ID	*E. flavescens*
OST-H-111E-9	Sewage	Robstown WWTP	3/10/2010	8/6/2010	Genus ID	*E. gallinarum*
OST-H-111E-10	Sewage	Robstown WWTP	3/10/2010	8/6/2010	Genus ID	*E. gallinarum*
OST-H-111E-11	Sewage	Robstown WWTP	3/10/2010	8/6/2010	Species ID	*E. faecium*
OST-H-111E-12	Sewage	Robstown WWTP	3/10/2010	8/6/2010	Species ID	*E. casseliflavus*
OST-H-112E-3	Sewage	Robstown WWTP	3/10/2010	4/29/2010	Species ID	*E. casseliflavus*
OST-H-112E-6	Sewage	Robstown WWTP	3/10/2010	4/29/2010	Species ID	*E. casseliflavus*
OST-H-112E-7	Sewage	Robstown WWTP	3/10/2010	4/29/2010	Species ID	*E. casseliflavus*
OST-H-112E-9	Sewage	Robstown WWTP	3/10/2010	4/29/2010	Species ID	*E. gallinarum*
OST-H-112E-11	Sewage	Robstown WWTP	3/10/2010	4/29/2010	Species ID	*E. gallinarum*
OST-H-113E-1	Sewage	Robstown WWTP	4/14/2010	8/6/2010	Genus ID	*E. mundtii*
OST-H-113E-4	Sewage	Robstown WWTP	4/14/2010	8/6/2010	Species ID	*E. faecium*
OST-H-113E-5	Sewage	Robstown WWTP	4/14/2010	8/6/2010	Species ID	*E. faecium*
OST-H-113E-6	Sewage	Robstown WWTP	4/14/2010	8/6/2010	Genus ID	*E. faecium*
OST-H-113E-7	Sewage	Robstown WWTP	4/14/2010	8/6/2010	Species ID	*E. mundtii*
OST-H-113E-10	Sewage	Robstown WWTP	4/14/2010	8/6/2010	Species ID	*E. mundtii*
OST-H-113E-12	Sewage	Robstown WWTP	4/14/2010	8/6/2010	Species ID	*E. mundtii*
OST-H-114E-1	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. flavescens*
OST-H-114E-2	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. flavescens*
OST-H-114E-3	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. gallinarum*
OST-H-114E-5	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. casseliflavus*
OST-H-114E-6	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. casseliflavus*
OST-H-114E-7	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. gallinarum*
OST-H-114E-10	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. casseliflavus*
OST-H-114E-13	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. casseliflavus*
OST-H-114E-14	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. gallinarum*
OST-H-114E-15	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-H-115E-1	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. flavescens*
OST-H-115E-2	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. flavescens*
OST-H-115E-3	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. casseliflavus*
OST-H-115E-4	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. flavescens*
OST-H-115E-6	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. flavescens*
OST-H-115E-7	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. casseliflavus*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-H-115E-9	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. casseliflavus*
OST-H-115E-10	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. pseudoavium*
OST-H-115E-11	Sewage	Robstown WWTP	8/24/2010	10/19/2010	Genus ID	*E. avium*
OST-H-115E-11	Sewage	Robstown WWTP	8/24/2010	10/19/2010	Species ID	*E. pseudoavium*
OST-H-115E-13	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. flavescens*
OST-H-115E-14	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. pseudoavium*
OST-H-115E-15	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. casseliflavus*
OST-H-116E-1	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. flavescens*
OST-H-116E-2	Sewage	Robstown WWTP	8/24/2010	10/7/2010	Species ID	*E. gallinarum*
OST-H-116E-3	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Genus ID	*E. casseliflavus*
OST-H-116E-4	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. casseliflavus*
OST-H-116E-5	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. casseliflavus*
OST-H-116E-6	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-H-116E-7	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Genus ID	*E. mundtii*
OST-H-116E-8	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. pseudoavium*
OST-H-116E-9	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. casseliflavus*
OST-H-116E-10	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-H-116E-12	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. casseliflavus*
OST-H-116E-14	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-H-116E-15	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Genus ID	*E. avium*
OST-H-116E-16	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Genus ID	*E. avium*
OST-H-117I-1	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-H-117I-2	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Genus ID	*E. mundtii*
OST-H-117I-3	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-H-117I-4	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-H-117I-5	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-H-117I-6	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-H-117I-7	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. mundtii*
OST-H-117I-9	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Genus ID	*E. gallinarum*
OST-H-117I-11	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Genus ID	*E. mundtii*
OST-H-117I-13	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. faecalis*
OST-H-117I-16	Sewage	Robstown WWTP	8/24/2010	9/30/2010	Species ID	*E. gallinarum*
OST-H-114E-2	Sewage	Robstown WWTP	8/24/2010	4/6/2011	Species ID	*E.gallinarum*
OST-H-118E-1	Sewage	Robstown WWTP	3/4/2011	4/6/2011	Species ID	*E.gallinarum*
OST-H-118E-3	Sewage	Robstown WWTP	3/4/2011	4/6/2011	Genus ID	*E.faecium*
OST-H-118E-4	Sewage	Robstown WWTP	3/4/2011	4/6/2011	Genus ID	*E.mundtii*
OST-H-118E-5	Sewage	Robstown WWTP	3/4/2011	4/6/2011	Species ID	*E.gallinarum*
OST-H-119E-1	Sewage	Robstown WWTP	3/4/2011	4/6/2011	Species ID	*E.flavescens*
OST-H-120E-1	Sewage	Robstown WWTP	1/26/2011	3/29/2011	Species ID	*E.gallinarum*
OST-H-120E-2	Sewage	Robstown WWTP	1/26/2011	3/29/2011	Species ID	*E.mundtii*
OST-H-120E-3	Sewage	Robstown WWTP	1/26/2011	3/29/2011	Species ID	*E.gallinarum*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-H-120E-4	Sewage	Robstown WWTP	1/26/2011	3/29/2011	Genus ID	*E.mundtii*
OST-H-121E-1	Sewage	Robstown WWTP	1/26/2011	3/29/2011	Species ID	*E.flavescens*
OST-H-121E-2	Sewage	Robstown WWTP	1/26/2011	3/29/2011	Species ID	*E.mundtii*
OST-H-121E-3	Sewage	Robstown WWTP	1/26/2011	3/29/2011	Species ID	*E.durans*
OST-H-121E-4	Sewage	Robstown WWTP	1/26/2011	3/29/2011	Species ID	*E.gallinarum*
OST-D-101-1	Canine	Nueces Veterinary Hospital	1/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-D-101-2	Canine	Nueces Veterinary Hospital	1/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-D-101-3	Canine	Nueces Veterinary Hospital	1/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-D-101-4	Canine	Nueces Veterinary Hospital	1/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-D-101-6	Canine	Nueces Veterinary Hospital	1/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-D-101-8	Canine	Nueces Veterinary Hospital	1/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-D-101-9	Canine	Nueces Veterinary Hospital	1/14/2010	9/29/2010	Species ID	*E. faecalis*
OST-D-102-2	Canine	Nueces Veterinary Hospital	1/14/2010	7/14/2010	Species ID	*E. faecium*
OST-D-102-3	Canine	Nueces Veterinary Hospital	1/14/2010	7/14/2010	Species ID	*E. gallinarum*
OST-D-102-4	Canine	Nueces Veterinary Hospital	1/14/2010	7/14/2010	Species ID	*E. faecium*
OST-D-102-5	Canine	Nueces Veterinary Hospital	1/14/2010	7/14/2010	Genus ID	*E. gallinarum*
OST-D-102-6	Canine	Nueces Veterinary Hospital	1/14/2010	7/14/2010	Species ID	*E. faecium*
OST-D-102-7	Canine	Nueces Veterinary Hospital	1/14/2010	7/14/2010	Species ID	*E. faecium*
OST-D-102-8	Canine	Nueces Veterinary Hospital	1/14/2010	7/14/2010	Species ID	*E. faecalis*
OST-D-102-9	Canine	Nueces Veterinary Hospital	1/14/2010	7/14/2010	Genus ID	*E. faecium*
OST-D-105-1	Canine	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. faecium*
OST-D-105-2	Canine	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. faecium*
OST-D-105-3	Canine	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. faecium*
OST-D-105-4	Canine	Marvin Prewitt Residence	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-105-6	Canine	Marvin Prewitt Residence	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-105-7	Canine	Marvin Prewitt Residence	2/26/2010	7/14/2010	Species ID	*E. hirae*
OST-D-105-8	Canine	Marvin Prewitt Residence	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-105-9	Canine	Marvin Prewitt Residence	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-105-10	Canine	Marvin Prewitt Residence	2/26/2010	7/14/2010	Genus ID	*E. hirae*
OST-D-105-11	Canine	Marvin Prewitt Residence	2/26/2010	7/14/2010	Genus ID	*E. faecium*
OST-D-105-12	Canine	Marvin Prewitt Residence	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-106-1	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Genus ID	*E. faecium*
OST-D-106-2	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Genus ID	*E. faecium*
OST-D-106-3	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-106-4	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-106-8	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Genus ID	*E. faecium*
OST-D-108-1	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-108-2	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-108-3	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-108-4	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. mundtii*
OST-D-108-5	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. faecium*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-D-108-6	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. mundtii*
OST-D-108-7	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. mundtii*
OST-D-108-10	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-108-11	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-108-12	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-109-1	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-109-2	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. gallinarum*
OST-D-109-3	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Genus ID	*E. gallinarum*
OST-D-109-4	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-109-7	Canine	Gulf Coast Animal Shelter	2/26/2010	8/6/2010	Species ID	*E. gallinarum*
OST-D-109-8	Canine	Gulf Coast Animal Shelter	2/26/2010	8/6/2010	Species ID	*E. faecium*
OST-D-109-9	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. faecium*
OST-D-109-10	Canine	Gulf Coast Animal Shelter	2/26/2010	8/6/2010	Species ID	*E. faecium*
OST-D-109-11	Canine	Gulf Coast Animal Shelter	2/26/2010	8/6/2010	Species ID	*E. faecalis*
OST-D-109-12	Canine	Gulf Coast Animal Shelter	2/26/2010	7/14/2010	Species ID	*E. gallinarum*
OST-D-114-1	Canine	Eileen Rogers Pasture	4/14/2010	7/14/2010	Species ID	*E. faecalis*
OST-D-115-1	Cockerspaniel Mix (Canine)	Nueces County Animal Control	8/24/2010	10/7/2010	Species ID	*E. faecium*
OST-D-115-2	Cockerspaniel Mix (Canine)	Nueces County Animal Control	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-D-115-3	Cockerspaniel Mix (Canine)	Nueces County Animal Control	8/24/2010	10/7/2010	Species ID	*E. faecium*
OST-D-115-5	Cockerspaniel Mix (Canine)	Nueces County Animal Control	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-D-115-6	Cockerspaniel Mix (Canine)	Nueces County Animal Control	8/24/2010	10/7/2010	Species ID	*E. faecium*
OST-D-115-8	Cockerspaniel Mix (Canine)	Nueces County Animal Control	8/24/2010	10/7/2010	Species ID	*E. faecalis*
OST-D-115-9	Cockerspaniel Mix (Canine)	Nueces County Animal Control	8/24/2010	10/7/2010	Species ID	*E. faecium*
OST-D-115-10	Cockerspaniel Mix (Canine)	Nueces County Animal Control	8/24/2010	10/19/2010	Species ID	*E. faecium*
OST-D-115-11	Cockerspaniel Mix (Canine)	Nueces County Animal Control	8/24/2010	10/19/2010	Species ID	*E. faecium*
OST-D-115-12	Cockerspaniel Mix (Canine)	Nueces County Animal Control	8/24/2010	10/7/2010	Species ID	*E. faecium*
OST-D-115-13	Cockerspaniel Mix (Canine)	Nueces County Animal Control	8/24/2010	10/7/2010	Species ID	*E. faecium*
OST-D-115-14	Cockerspaniel Mix (Canine)	Nueces County Animal Control	8/24/2010	10/7/2010	Species ID	*E. faecium*
OST-D-115-15	Cockerspaniel Mix (Canine)	Nueces County Animal Control	8/24/2010	10/19/2010	Species ID	*E. faecium*
OST-D-115-16	Cockerspaniel Mix (Canine)	Nueces County Animal Control	8/24/2010	10/7/2010	Species ID	*E. faecium*
OST-E-100-1	Horse	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. hirae*
OST-E-100-2	Horse	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. flavescens*
OST-E-100-3	Horse	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. faecium*
OST-E-100-5	Horse	Marvin Prewitt Residence	2/26/2010	8/6/2010	Genus ID	*E. gallinarum*
OST-E-100-6	Horse	Marvin Prewitt Residence	2/26/2010	8/6/2010	Genus ID	*E. solitarius*
OST-E-100-7	Horse	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. gallinarum*
OST-E-100-8	Horse	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. gallinarum*
OST-E-100-9	Horse	Marvin Prewitt Residence	2/26/2010	8/6/2010	Genus ID	*E. mundtii*
OST-E-100-10	Horse	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. mundtii*
OST-E101-1	Horse	Rodeo Run Arena - Stable 1	8/25/2010	10/21/2010	Species ID	*E. faecalis*
OST-E-102-1	Horse	Rodeo Run Arena - Stable 1	8/25/2010	10/19/2010	Genus ID	*E. mundtii*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-E-102-2	Horse	Rodeo Run Arena - Stable 1	8/25/2010	10/19/2010	Species ID	*E. casseliflavus*
OST-E102-3	Horse	Rodeo Run Arena - Stable 1	8/25/2010	10/21/2010	Species ID	*E. mundtii*
OST-E-102-6	Horse	Rodeo Run Arena - Stable 1	8/25/2010	10/19/2010	Species ID	*E. flavescens*
OST-E-104-2	Horse	Rodeo Run Arena - Stable 3	8/25/2010	10/26/2010	Species ID	*E. faecalis*
OST-E-105-1	Horse	Rodeo Run Arena - Stable 15	8/25/2010	10/26/2010	Species ID	*E. mundtii*
OST-E-105-2	Horse	Rodeo Run Arena - Stable 15	8/25/2010	10/26/2010	Species ID	*E. mundtii*
OST-E-105-3	Horse	Rodeo Run Arena - Stable 15	8/25/2010	10/26/2010	Species ID	*E. mundtii*
OST-E-105-4	Horse	Rodeo Run Arena - Stable 15	8/25/2010	10/26/2010	Species ID	*E. mundtii*
OST-E-105-7	Horse	Rodeo Run Arena - Stable 15	8/25/2010	10/26/2010	Species ID	*E. mundtii*
OST-E-107-3	Horse	Rodeo Run Arena - Stable 16	8/25/2010	10/26/2010	Species ID	*E. flavescens*
OST-E-107-5	Horse	Rodeo Run Arena - Stable 16	8/25/2010	10/26/2010	Species ID	*E. mundtii*
OST-E-107-6	Horse	Rodeo Run Arena - Stable 16	8/25/2010	10/26/2010	Species ID	*E. gallinarum*
OST-E-107-8	Horse	Rodeo Run Arena - Stable 16	8/25/2010	10/26/2010	Species ID	*E. casseliflavus*
OST-E-107-9	Horse	Rodeo Run Arena - Stable 16	8/25/2010	10/26/2010	Species ID	*E. faecium*
OST-E-107-10	Horse	Rodeo Run Arena - Stable 16	8/25/2010	10/26/2010	Species ID	*E. faecium*
OST-E-109-8	Horse	Rodeo Run Arena - Stable 6	8/25/2010	10/26/2010	Species ID	*E. casseliflavus*
OST-E-110-2	Horse	Rodeo Run Arena - Open Pasture	8/25/2010	10/26/2010	Species ID	*E. faecalis*
OST-E-110-4	Horse	Rodeo Run Arena - Open Pasture	8/25/2010	10/26/2010	Species ID	*E. faecalis*
OST-E-110-8	Horse	Rodeo Run Arena - Open Pasture	8/25/2010	10/26/2010	Species ID	*E. faecalis*
OST-E-111-1	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. flavescens*
OST-E-111-2	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. flavescens*
OST-E-111-3	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. flavescens*
OST-E-111-4	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. mundtii*
OST-E-111-5	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Genus ID	*E. flavescens*
OST-E-111-6	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. flavescens*
OST-E-111-7	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. casseliflavus*
OST-E-111-8	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. flavescens*
OST-E-111-9	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. faecalis*
OST-E-111-10	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. flavescens*
OST-E-111-11	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. casseliflavus*
OST-E-111-12	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. gallinarum*
OST-E-111-13	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. casseliflavus*
OST-E-111-14	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. flavescens*
OST-E-111-15	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. flavescens*
OST-E-111-16	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. faecalis*
OST-E-111-17	Horse	Rodeo Run Arena - Stable 7	8/25/2010	10/26/2010	Species ID	*E. gallinarum*
OST-E-115-1	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/26/2010	Species ID	*E. mundtii*
OST-E-115-2	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Genus ID	*E. mundtii*
OST-E-115-6	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Genus ID	*E. faecium*
OST-E-115-7	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/26/2010	Species ID	*E. mundtii*
OST-E-115-8	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Genus ID	*E. faecium*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-E-115-14	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/19/2010	Genus ID	*E. mundtii*
OST-E-115-15	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/19/2010	Species ID	*E. faecium*
OST-E-117-1	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. faecalis*
OST-E-117-2	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. faecium*
OST-E-117-7	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. faecalis*
OST-E-117-12	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. faecalis*
OST-E-118-5	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. faecalis*
OST-E-118-6	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. faecalis*
OST-E-118-7	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. faecalis*
OST-E-118-11	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. faecalis*
OST-E-118-12	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. faecalis*
OST-E-119-4	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/19/2010	Species ID	*E. faecium*
OST-E-119-5	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/19/2010	Species ID	*E. mundtii*
OST-E-119-7	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/19/2010	Species ID	*E. mundtii*
OST-E-119-9	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/19/2010	Species ID	*E. mundtii*
OST-E-119-10	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/19/2010	Species ID	*E. mundtii*
OST-E-119-11	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/19/2010	Species ID	*E. gallinarum*
OST-E-119-13	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/19/2010	Species ID	*E. gallinarum*
OST-E-120-2	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/19/2010	Species ID	*E. faecium*
OST-E-120-3	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/19/2010	Species ID	*E. faecium*
OST-E-120-11	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/19/2010	Species ID	*E. faecium*
OST-E-120-13	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/26/2010	Species ID	*E. faecium*
OST-E-120-14	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/26/2010	Species ID	*E. faecium*
OST-E-120-15	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/26/2010	Genus ID	*E. dispar*
OST-E-120-16	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/26/2010	Species ID	*E. faecium*
OST-E-121-4	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/26/2010	Species ID	*E. solitarius*
OST-E-121-7	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/26/2010	Species ID	*E. faecium*
OST-E-121-9	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/26/2010	Species ID	*E. faecium*
OST-E-122-1	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/26/2010	Species ID	*E. flavescens*
OST-E-122-2	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/26/2010	Species ID	*E. casseliflavus*
OST-E-122-5	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. faecalis*
OST-E-122-10	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/21/2010	Species ID	*E. flavescens*
OST-E-123-2	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/26/2010	Species ID	*E. faecium*
OST-E-123-4	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. hirae*
OST-E-123-5	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. faecalis*
OST-E-123-6	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. gallinarum*
OST-E-123-7	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. hirae*
OST-E-123-8	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. faecium*
OST-E-123-9	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. faecium*
OST-E-123-10	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. casseliflavus*
OST-E-123-11	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. casseliflavus*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-E-123-14	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Species ID	*E. gallinarum*
OST-E-123-18	Horse	Pee Wee's Animal Shelter - Field	8/25/2010	10/29/2010	Genus ID	*E. flavescens*
OST-E-124-1	Horse	Pat Walker Residence - 4104 FM 1694	1/26/2011	3/29/2011	Species ID	*E. casseliflavus*
OST-E-124-1	Horse	Pat Walker Residence - 4104 FM 1694	1/26/2011	4/6/2011	Species ID	*E. flavescens*
OST-E-124-2	Horse	Pat Walker Residence - 4104 FM 1694	1/26/2011	4/6/2011	Species ID	*E. mundtii*
OST-E-126-1	Horse	Pat Walker Residence - 4104 FM 1694	1/26/2011	3/29/2011	Species ID	*E. casseliflavus*
OST-E-126-2	Horse	Pat Walker Residence - 4104 FM 1694	1/26/2011	3/29/2011	Species ID	*E. flavescens*
OST-E-129-4	Horse	Havelkas Residence - 3727 Co Rd61	1/26/2011	3/31/2011	Species ID	*E. flavescens*
OST-E-129-5	Horse	Havelkas Residence - 3727 Co Rd61	1/26/2011	3/31/2011	Species ID	*E. casseliflavus*
OST-E-129-6	Horse	Havelkas Residence - 3727 Co Rd61	1/26/2011	3/31/2011	Species ID	*E. casseliflavus*
OST-E-131-1	Horse	Havelkas Residence - 3727 Co Rd61	1/26/2011	3/31/2011	Genus ID	*E. casseliflavus*
OST-E-131-2	Horse	Havelkas Residence - 3727 Co Rd61	1/26/2011	3/31/2011	Species ID	*E. flavescens*
OST-E-131-3	Horse	Havelkas Residence - 3727 Co Rd61	1/26/2011	3/31/2011	Genus ID	*E. gallinarum*
OST-E-131-4	Horse	Havelkas Residence - 3727 Co Rd61	1/26/2011	3/31/2011	Species ID	*E. flavescens*
OST-E-131-5	Horse	Havelkas Residence - 3727 Co Rd61	1/26/2011	3/31/2011	Species ID	*E. hirae*
OST-E-131-6	Horse	Havelkas Residence - 3727 Co Rd61	1/26/2011	3/31/2011	Genus ID	*E. casseliflavus*
OST-W-100-11	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. casseliflavus*
OST-W-100-12	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. mundtii*
OST-W-100-2	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. mundtii*
OST-W-100-3	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. faecium*
OST-W-100-5	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. casseliflavus*
OST-W-100-6	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Genus ID	*E. flavescens*
OST-W-100-7	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. casseliflavus*
OST-W-100-8	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. flavescens*
OST-W-100-9	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. mundtii*
OST-W-102-1	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. hirae*
OST-W-102-11	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. casseliflavus*
OST-W-102-3	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. mundtii*
OST-W-102-6	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. faecium*
OST-W-103-1	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. casseliflavus*
OST-W-103-10	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Genus ID	*E. gallinarum*
OST-W-103-11	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Genus ID	*E. casseliflavus*
OST-W-103-12	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. mundtii*
OST-W-103-2	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. mundtii*
OST-W-103-5	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. gallinarum*
OST-W-103-6	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. casseliflavus*
OST-W-103-7	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. hirae*
OST-W-103-8	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Genus ID	*E. hirae*
OST-W-103-9	Sheep	Marvin Prewitt Residence	2/26/2010	8/6/2010	Species ID	*E. mundtii*
OST-W-104-1	Skunk	8600 South Staples	2/26/2010	8/6/2010	Species ID	*E. faecalis*
OST-W-104-10	Skunk	8600 South Staples	2/26/2010	8/6/2010	Species ID	*E. faecalis*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-W-104-12	Skunk	8600 South Staples	2/26/2010	8/6/2010	Species ID	*E. faecalis*
OST-W-104-2	Skunk	8600 South Staples	2/26/2010	8/6/2010	Species ID	*E. faecalis*
OST-W-104-3	Skunk	8600 South Staples	2/26/2010	8/6/2010	Species ID	*E. faecalis*
OST-W-104-4	Skunk	8600 South Staples	2/26/2010	8/6/2010	Species ID	*E. faecalis*
OST-W-104-5	Skunk	8600 South Staples	2/26/2010	8/6/2010	Species ID	*E. faecalis*
OST-W-104-6	Skunk	8600 South Staples	2/26/2010	8/6/2010	Species ID	*E. faecalis*
OST-W-104-7	Skunk	8600 South Staples	2/26/2010	8/6/2010	Species ID	*E. faecalis*
OST-W-104-8	Skunk	8600 South Staples	2/26/2010	8/6/2010	Species ID	*E. faecalis*
OST-W-104-9	Skunk	8600 South Staples	2/26/2010	8/6/2010	Species ID	*E. faecalis*
OST-W-114-1	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-10	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-11	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-12	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-13	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-14	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-15	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-16	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-17	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-19	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-2	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-20	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-3	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-4	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-5	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-6	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-8	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-114-9	Skunk	Right before TCEQ - 18499 Bridge	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-1	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-10	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-11	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-12	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-14	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-15	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-16	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-17	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-18	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-19	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-2	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-20	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-3	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-4	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-W-115-5	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-6	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-7	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-8	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-115-9	Skunk	7833 FM 665 - Near Memorial Gardens	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-10	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Genus ID	*E. faecium*
OST-W-116-11	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-12	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-14	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-15	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-16	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-17	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-W-116-18	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-19	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-2	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-20	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-3	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-4	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-5	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. mundtii*
OST-W-116-6	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-7	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-8	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-116-9	Unknown - Road Kill	7636 Weber Rd. Near TCEQ - GW06	9/9/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-117-10	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/29/2010	Species ID	*E. faecalis*
OST-W-117-11	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/29/2010	Species ID	*E. faecalis*
OST-W-117-13	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-117-15	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-117-16	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-117-17	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-117-18	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-117-19	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-117-20	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-117-3	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/29/2010	Species ID	*E. faecalis*
OST-W-117-4	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/29/2010	Species ID	*E. faecalis*
OST-W-117-5	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/29/2010	Species ID	*E. faecalis*
OST-W-117-6	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/29/2010	Species ID	*E. faecalis*
OST-W-117-8	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/29/2010	Species ID	*E. faecalis*
OST-W-117-9	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/29/2010	Species ID	*E. faecalis*
OST-W-118-1	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-118-11	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/29/2010	Species ID	*E. faecalis*
OST-W-118-12	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/29/2010	Species ID	*E. faecalis*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-W-118-13	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	11/10/2010	Species ID	*E. faecalis*
OST-W-118-14	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/29/2010	Species ID	*E. faecalis*
OST-W-118-15	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/29/2010	Species ID	*E. faecalis*
OST-W-118-17	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-118-18	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-118-2	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-118-3	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-118-4	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-118-8	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/29/2010	Species ID	*E. faecalis*
OST-W-118-9	Racoon	Memorial Garden Cemetary - TCEQ 18500	9/10/2010	10/29/2010	Species ID	*E. faecalis*
OST-W-119-1	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. casseliflavus*
OST-W-119-10	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-119-11	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-119-13	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. mundtii*
OST-W-119-15	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Genus ID	*E. gallinarum*
OST-W-119-16	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. mundtii*
OST-W-119-17	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. hirae*
OST-W-119-18	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. gallinarum*
OST-W-119-2	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-119-20	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. mundtii*
OST-W-119-3	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-119-4	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. mundtii*
OST-W-119-4	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-119-5	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-119-6	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-119-7	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-119-8	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-119-9	Racoon	Railroad Bridge - TCEQ 18499	9/13/2010	10/26/2010	Species ID	*E. faecalis*
OST-W-120-1	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.gallinarum*
OST-W-120-2	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.gallinarum*
OST-W-120-3	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.hirae*
OST-W-120-4	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.faecalis*
OST-W-120-5	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.faecalis*
OST-W-121-1	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.faecalis*
OST-W-121-2	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.faecalis*
OST-W-121-2	Rabbit	Corpus Christi International Airport	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-121-3	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.faecalis*
OST-W-121-4	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.casseliflavus*
OST-W-121-6	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.faecalis*
OST-W-122-1	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.faecalis*
OST-W-122-2	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.faecalis*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-W-122-3	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.faecalis*
OST-W-122-4	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.faecalis*
OST-W-122-5	Rabbit	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.faecalis*
OST-W-123-1	Racoon	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.flavescens*
OST-W-123-2	Racoon	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.flavescens*
OST-W-123-3	Racoon	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.casseliflavus*
OST-W-123-4	Racoon	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.flavescens*
OST-W-123-5	Racoon	Corpus Christi International Airport	12/20/2010	3/29/2011	Genus ID	*E.flavescens*
OST-W-124-1	Racoon	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.gallinarum*
OST-W-124-2	Racoon	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.flavescens*
OST-W-124-3	Racoon	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.flavescens*
OST-W-124-4	Racoon	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.flavescens*
OST-W-124-5	Racoon	Corpus Christi International Airport	12/20/2010	3/29/2011	Species ID	*E.flavescens*
OST-W-125-1	Racoon	Corpus Christi International Airport	12/20/2010	3/31/2011	Species ID	*E.gallinarum*
OST-W-125-2	Racoon	Corpus Christi International Airport	12/20/2010	3/31/2011	Genus ID	*E.faecium*
OST-W-125-4	Racoon	Corpus Christi International Airport	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-125-5	Racoon	Corpus Christi International Airport	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-126-1	Rabbit	Corpus Christi International Airport	12/20/2010	3/31/2011	Genus ID	*E.gallinarum*
OST-W-126-2	Rabbit	Corpus Christi International Airport	12/20/2010	3/31/2011	Genus ID	*E.gallinarum*
OST-W-126-3	Rabbit	Corpus Christi International Airport	12/20/2010	3/31/2011	Species ID	*E.casseliflavus*
OST-W-126-8	Rabbit	Corpus Christi International Airport	12/20/2010	3/31/2011	Genus ID	*E.gallinarum*
OST-W-127-1	Rabbit	Corpus Christi International Airport	12/20/2010	3/31/2011	Genus ID	*E.flavescens*
OST-W-127-2	Rabbit	Corpus Christi International Airport	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-127-3	Rabbit	Corpus Christi International Airport	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-127-4	Rabbit	Corpus Christi International Airport	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-127-5	Rabbit	Corpus Christi International Airport	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-128-1	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Genus ID	*E.faecium*
OST-W-128-2	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-128-3	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-128-4	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-128-5	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-129-1	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-129-2	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-130-2	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-130-3	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-130-5	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-131-1	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-131-5	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.flavescens*
OST-W-132-1	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.mundtii*
OST-W-132-2	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Genus ID	*E.hirae*
OST-W-132-3	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*

Isolate #	Source	Location	Date Collected	Date Analyzed	ID Result	*Genus/Species*
OST-W-132-5	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-133-1	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecium*
OST-W-133-3	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Genus ID	*E.faecium*
OST-W-133-4	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-134-1	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-134-2	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-134-3	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-134-4	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-134-5	Racoon	TCEQ Station 18499	12/20/2010	3/31/2011	Species ID	*E.faecalis*
OST-W-135-1	Racoon	Corpus Christi International Airport	1/26/2011	3/31/2011	Genus ID	*E.mundtii*
OST-W-135-2	Racoon	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.faecium*
OST-W-135-3	Racoon	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.faecium*
OST-W-135-6	Racoon	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.faecalis*
OST-W-136-1	Rabbit	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.gallinarum*
OST-W-136-2	Rabbit	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.gallinarum*
OST-W-136-3	Rabbit	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.gallinarum*
OST-W-136-4	Rabbit	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.gallinarum*
OST-W-136-5	Rabbit	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.gallinarum*
OST-W-137-1	Coyote	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.faecium*
OST-W-137-2	Coyote	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.mundtii*
OST-W-137-3	Coyote	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.gallinarum*
OST-W-137-4	Coyote	Corpus Christi International Airport	1/26/2011	3/31/2011	Genus ID	*E.mundtii*
OST-W-137-5	Coyote	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.mundtii*
OST-W-138-1	Racoon	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.faecalis*
OST-W-138-2	Racoon	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.faecalis*
OST-W-138-3	Racoon	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.faecalis*
OST-W-138-4	Racoon	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.faecalis*
OST-W-138-5	Racoon	Corpus Christi International Airport	1/26/2011	3/31/2011	Species ID	*E.faecalis*
OST-W-139-2	Coyote	Hwy 44 & Violet Intersection	1/26/2011	3/31/2011	Species ID	*E.faecium*
OST-W-139-3	Coyote	Hwy 44 & Violet Intersection	1/26/2011	3/31/2011	Species ID	*E.faecalis*
OST-W-139-4	Coyote	Hwy 44 & Violet Intersection	1/26/2011	3/31/2011	Species ID	*E.faecalis*
OST-W-139-5	Coyote	Hwy 44 & Violet Intersection	1/26/2011	3/31/2011	Species ID	*E.faecalis*
OST-W-139-7	Coyote	Hwy 44 & Violet Intersection	1/26/2011	3/31/2011	Species ID	*E.faecalis*
OST-W-146-8	Racoon	TCEQ Station 18499	3/4/2011	3/31/2011	Species ID	*E.casseliflavus*

Appendix F
Linear Discriminant Analysis Average Rates of Correct Classification Tables for Wet and Dry events, and for West Oso Creek and Main Oso Creek Discrimination

Appendix F Table 1: Discriminant analysis of wet event unknown source enterococci isolates compared to Oso Creek Library. Two-way model—human vs. non-human (equal prior probabilities)

Classification Results

			Predicted Group Membership		
		Type	Human	Non-Human	Total
Original	Count	Human	95	16	111
		Non-Human	65	909	974
		Unknowns	29	338	367
	%	Human	85.6	14.4	100.0
		Non-Human	6.7	93.3	100.0
		Unknowns	**7.9**	**92.1**	100.0

a. 92.5% of original grouped cases correctly classified.

Appendix F Table 2: Discriminant analysis of wet event unknown source enterococci isolates compared to Oso Creek Library. Seven-way model—human vs. cow vs. horse vs. dog vs. seagull vs. other avian vs. non-avian wildlife (equal prior probabilities)

Classification Results

			Predicted Group Membership							
		Type	Human	Cow	Horse	Dog	Seagull	Other Avian	Non-Avian Wildlife	Total
Original	Count	Human	86	3	3	3	0	14	2	111
		Cow	5	122	6	1	0	8	9	151
		Horse	5	7	64	2	0	6	8	92
		Dog	1	3	6	138	11	8	2	169
		Seagull	0	3	0	9	90	0	1	103
		Other Avian	9	7	23	4	0	199	34	276
		Non-Avian Wildlife	4	6	16	2	0	14	141	183
		Unknowns	18	48	72	10	1	75	143	367
	%	Human	77.5	2.7	2.7	2.7	.0	12.6	1.8	100.0
		Cow	3.3	80.8	4.0	.7	.0	5.3	6.0	100.0
		Horse	5.4	7.6	69.6	2.2	.0	6.5	8.7	100.0
		Dog	.6	1.8	3.6	81.7	6.5	4.7	1.2	100.0
		Seagull	.0	2.9	.0	8.7	87.4	.0	1.0	100.0
		Other Avian	3.3	2.5	8.3	1.4	.0	72.1	12.3	100.0
		Non-Avian Wildlife	2.2	3.3	8.7	1.1	.0	7.7	77.0	100.0
		Unknowns	4.9	13.1	19.6	2.7	.3	20.4	39.0	100.0

a. 77.4% of original grouped cases correctly classified.

Appendix F Table 3: Discriminant analysis of dry event unknown source enterococci isolates compared to Oso Creek Library. Two-way model—human vs. non-human (equal prior probabilities)

Classification Results

			Predicted Group Membership		
		Type	Human	Non-Human	Total
Original	Count	Human	95	16	111
		Non-Human	65	909	974
		Unknowns	36	389	425
	%	Human	85.6	14.4	100.0
		Non-Human	6.7	93.3	100.0
		Unknowns	**8.5**	**91.5**	100.0

a. 92.5% of original grouped cases correctly classified.

Appendix F Table 4: Discriminant analysis of dry event unknown source enterococci isolates compared to Oso Creek Library. Seven-way model—human vs. cow vs. horse vs. dog vs. seagull vs. other avian vs. non-avian wildlife (equal prior probabilities)

Classification Results[a]

			Predicted Group Membership							
		Type	Human	Cow	Horse	Dog	Seagull	Other Avian	Non-Avian Wildlife	Total
Original	Count	Human	86	3	3	3	0	14	2	111
		Cow	5	122	6	1	0	8	9	151
		Horse	5	7	64	2	0	6	8	92
		Dog	1	3	6	138	11	8	2	169
		Seagull	0	3	0	9	90	0	1	103
		Other Avian	9	7	23	4	0	199	34	276
		Non-Avian Wildlife	4	6	16	2	0	14	141	183
		Unknowns	17	58	77	17	2	167	87	425
	%	Human	77.5	2.7	2.7	2.7	.0	12.6	1.8	100.0
		Cow	3.3	80.8	4.0	.7	.0	5.3	6.0	100.0
		Horse	5.4	7.6	69.6	2.2	.0	6.5	8.7	100.0
		Dog	.6	1.8	3.6	81.7	6.5	4.7	1.2	100.0
		Seagull	.0	2.9	.0	8.7	87.4	.0	1.0	100.0
		Other Avian	3.3	2.5	8.3	1.4	.0	72.1	12.3	100.0
		Non-Avian Wildlife	2.2	3.3	8.7	1.1	.0	7.7	77.0	100.0
		Unknowns	**4.0**	**13.6**	**18.1**	**4.0**	**.5**	**39.3**	**20.5**	100.0

a. 77.4% of original grouped cases correctly classified.

Appendix F Table 5: Discriminant analysis of dry event unknown source enterococci isolates from West Oso Creek (station 18501) compared to Oso Creek Library. Two-way model—human vs. non-human (equal prior probabilities)

Classification Results

			Predicted Group Membership		
		Type	Human	Non-Human	Total
Original	Count	Human	95	16	111
		Non-Human	65	909	974
		Unknowns	12	138	150
	%	Human	85.6	14.4	100.0
		Non-Human	6.7	93.3	100.0
		Unknowns	**8.0**	**92.0**	100.0

a. 92.5% of original grouped cases correctly classified.

Appendix F Table 6: Discriminant analysis of dry event unknown source enterococci isolates from West Oso Creek (station 18501) compared to Oso Creek Library. Seven-way model—human vs. cow vs. horse vs. dog vs. seagull vs. other avian vs. wildlife (non-avian) (equal prior probabilities)

Classification Results

			Predicted Group Membership							
		Type	Human	Cow	Horse	Dog	Seagull	Other Avian	Non-Avian Wildlife	Total
Original	Count	Human	86	3	3	3	0	14	2	111
		Cow	5	122	6	1	0	8	9	151
		Horse	5	7	64	2	0	6	8	92
		Dog	1	3	6	138	11	8	2	169
		Seagull	0	3	0	9	90	0	1	103
		Other Avian	9	7	23	4	0	199	34	276
		Non-Avian Wildlife	4	6	16	2	0	14	141	183
		Unknowns	8	24	28	8	0	54	28	150
	%	Human	77.5	2.7	2.7	2.7	.0	12.6	1.8	100.0
		Cow	3.3	80.8	4.0	.7	.0	5.3	6.0	100.0
		Horse	5.4	7.6	69.6	2.2	.0	6.5	8.7	100.0
		Dog	.6	1.8	3.6	81.7	6.5	4.7	1.2	100.0
		Seagull	.0	2.9	.0	8.7	87.4	.0	1.0	100.0
		Other Avian	3.3	2.5	8.3	1.4	.0	72.1	12.3	100.0
		Non-Avian Wildlife	2.2	3.3	8.7	1.1	.0	7.7	77.0	100.0
		Unknowns	**5.3**	**16.0**	**18.7**	**5.3**	**.0**	**36.0**	**18.7**	100.0

a. 77.4% of original grouped cases correctly classified.

Appendix F Table 7: Discriminant analysis of dry event unknown source enterococci isolates from Main Oso Creek (stations 18499, 18500, and 20559) compared to Oso Creek Library. Two-way model—human vs. non-human (equal prior probabilities)

Classification Results

			Predicted Group Membership		
		Type	Human	Non-Human	Total
Original	Count	Human	95	16	111
		Non-Human	65	909	974
		Unknowns	30	320	350
	%	Human	85.6	14.4	100.0
		Non-Human	6.7	93.3	100.0
		Unknowns	**8.6**	**91.4**	100.0

a. 92.5% of original grouped cases correctly classified.

Appendix F Table 8: Discriminant analysis of dry event unknown source enterococci isolates from Main Oso Creek (stations 18499, 18500, and 20559) compared to Oso Creek Library. Seven-way model—human vs. cow vs. horse vs. dog vs. seagull vs. other avian vs. non-avian wildlife (equal prior probabilities)

Classification Results

			Predicted Group Membership							
		Type	Human	Cow	Horse	Dog	Seagull	Other Avian	Non-Avian Wildlife	Total
Original	Count	Human	86	3	3	3	0	14	2	111
		Cow	5	122	6	1	0	8	9	151
		Horse	5	7	64	2	0	6	8	92
		Dog	1	3	6	138	11	8	2	169
		Seagull	0	3	0	9	90	0	1	103
		Other Avian	9	7	23	4	0	199	34	276
		Non-Avian Wildlife	4	6	16	2	0	14	141	183
		Unknowns	13	46	63	13	2	140	73	350
	%	Human	77.5	2.7	2.7	2.7	.0	12.6	1.8	100.0
		Cow	3.3	80.8	4.0	.7	.0	5.3	6.0	100.0
		Horse	5.4	7.6	69.6	2.2	.0	6.5	8.7	100.0
		Dog	.6	1.8	3.6	81.7	6.5	4.7	1.2	100.0
		Seagull	.0	2.9	.0	8.7	87.4	.0	1.0	100.0
		Other Avian	3.3	2.5	8.3	1.4	.0	72.1	12.3	100.0
		Non-Avian Wildlife	2.2	3.3	8.7	1.1	.0	7.7	77.0	100.0
		Unknowns	**3.7**	**13.1**	**18.0**	**3.7**	**.6**	**40.0**	**20.9**	100.0

a. 77.4% of original grouped cases correctly classified.

Appendix F Table 9: Discriminant analysis of wet event unknown source enterococci isolates from West Oso Creek (stations 18501 and 20198) compared to Oso Creek Library. Two-way model—human vs. non-human (equal prior probabilities)

Classification Results

			Predicted Group Membership		
		Type	Human	Non-Human	Total
Original	Count	Human	95	16	111
		Non-Human	65	909	974
		Unknowns	15	180	195
	%	Human	85.6	14.4	100.0
		Non-Human	6.7	93.3	100.0
		Unknowns	**7.7**	**92.3**	100.0

a. 92.5% of original grouped cases correctly classified.

Appendix F Table 10: Discriminant analysis of wet event unknown source enterococci isolates from West Oso Creek (stations 18501 and 20198) compared to Oso Creek Library. Seven-way model—human vs. cow vs. horse vs. dog vs. seagull vs. other avian vs. non-avian wildlife (equal prior probabilities)

Classification Results

			Predicted Group Membership							
		Type	Human	Cow	Horse	Dog	Seagull	Other Avian	Non-Avian Wildlife	Total
Original	Count	Human	86	3	3	3	0	14	2	111
		Cow	5	122	6	1	0	8	9	151
		Horse	5	7	64	2	0	6	8	92
		Dog	1	3	6	138	11	8	2	169
		Seagull	0	3	0	9	90	0	1	103
		Other Avian	9	7	23	4	0	199	34	276
		Non-Avian Wildlife	4	6	16	2	0	14	141	183
		Unknowns	9	24	44	1	0	29	88	195
	%	Human	77.5	2.7	2.7	2.7	.0	12.6	1.8	100.0
		Cow	3.3	80.8	4.0	.7	.0	5.3	6.0	100.0
		Horse	5.4	7.6	69.6	2.2	.0	6.5	8.7	100.0
		Dog	.6	1.8	3.6	81.7	6.5	4.7	1.2	100.0
		Seagull	.0	2.9	.0	8.7	87.4	.0	1.0	100.0
		Other Avian	3.3	2.5	8.3	1.4	.0	72.1	12.3	100.0
		Non-Avian Wildlife	2.2	3.3	8.7	1.1	.0	7.7	77.0	100.0
		Unknowns	**4.6**	**12.3**	**22.6**	**.5**	**.0**	**14.9**	**45.1**	100.0

a. 77.4% of original grouped cases correctly classified.

Appendix F Table 11: Discriminant analysis of wet event unknown source enterococci isolates from Main Oso Creek (stations 18499, 18500, and 20559) compared to Oso Creek Library. Two-way model—human vs. non-human (equal prior probabilities)

Classification Results

			Predicted Group Membership		
		Type	Human	Non-Human	Total
Original	Count	Human	95	16	111
		Non-Human	65	909	974
		Unknowns	14	158	172
	%	Human	85.6	14.4	100.0
		Non-Human	6.7	93.3	100.0
		Unknowns	**8.1**	**91.9**	100.0

a. 92.5% of original grouped cases correctly classified.

Appendix F Table 12: Discriminant analysis of wet event unknown source enterococci isolates from Main Oso Creek (stations 18499, 18500, and 20559) compared to Oso Creek Library. Seven-way model—human vs. cow vs. horse vs. dog vs. seagull vs. other avian vs. non-avian wildlife (equal prior probabilities)

Classification Results

			Predicted Group Membership							
		Type	Human	Cow	Horse	Dog	Seagull	Other Avian	Non-Avian Wildlife	Total
Original	Count	Human	86	3	3	3	0	14	2	111
		Cow	5	122	6	1	0	8	9	151
		Horse	5	7	64	2	0	6	8	92
		Dog	1	3	6	138	11	8	2	169
		Seagull	0	3	0	9	90	0	1	103
		Other-Avian	9	7	23	4	0	199	34	276
		Non-Avian Wildlife	4	6	16	2	0	14	141	183
		Unknowns	9	24	28	9	1	46	55	172
	%	Human	77.5	2.7	2.7	2.7	.0	12.6	1.8	100.0
		Cow	3.3	80.8	4.0	.7	.0	5.3	6.0	100.0
		Horse	5.4	7.6	69.6	2.2	.0	6.5	8.7	100.0
		Dog	.6	1.8	3.6	81.7	6.5	4.7	1.2	100.0
		Seagull	.0	2.9	.0	8.7	87.4	.0	1.0	100.0
		Other-Avian	3.3	2.5	8.3	1.4	.0	72.1	12.3	100.0
		Non-Avian Wildlife	2.2	3.3	8.7	1.1	.0	7.7	77.0	100.0
		Unknowns	**5.2**	**14.0**	**16.3**	**5.2**	**.6**	**26.7**	**32.0**	100.0

a. 77.4% of original grouped cases correctly classified.