The background of the slide is a photograph of a river with a rocky bank on the left and a blue sky on the right. A large, rounded rectangular box with a cyan background and a black border is centered on the left side of the image. Inside this box, the title text is displayed in black. A smaller, rounded rectangular box with a light blue background and a black border is positioned below the main title box, containing the author and mentor information in black text.

**Environmental Analysis of
Local Recreational Waters for
the Presence of *Escherichia
coli O157:H7* and other
Coliform Bacteria**

Thuan D. Dao
Mentor: Monica A. Kingsley
*Texas Department of State
Health Services*

Project Collaborators

- **Thuan Duc Dao**

Concentration: Microbiology

Standing: Senior

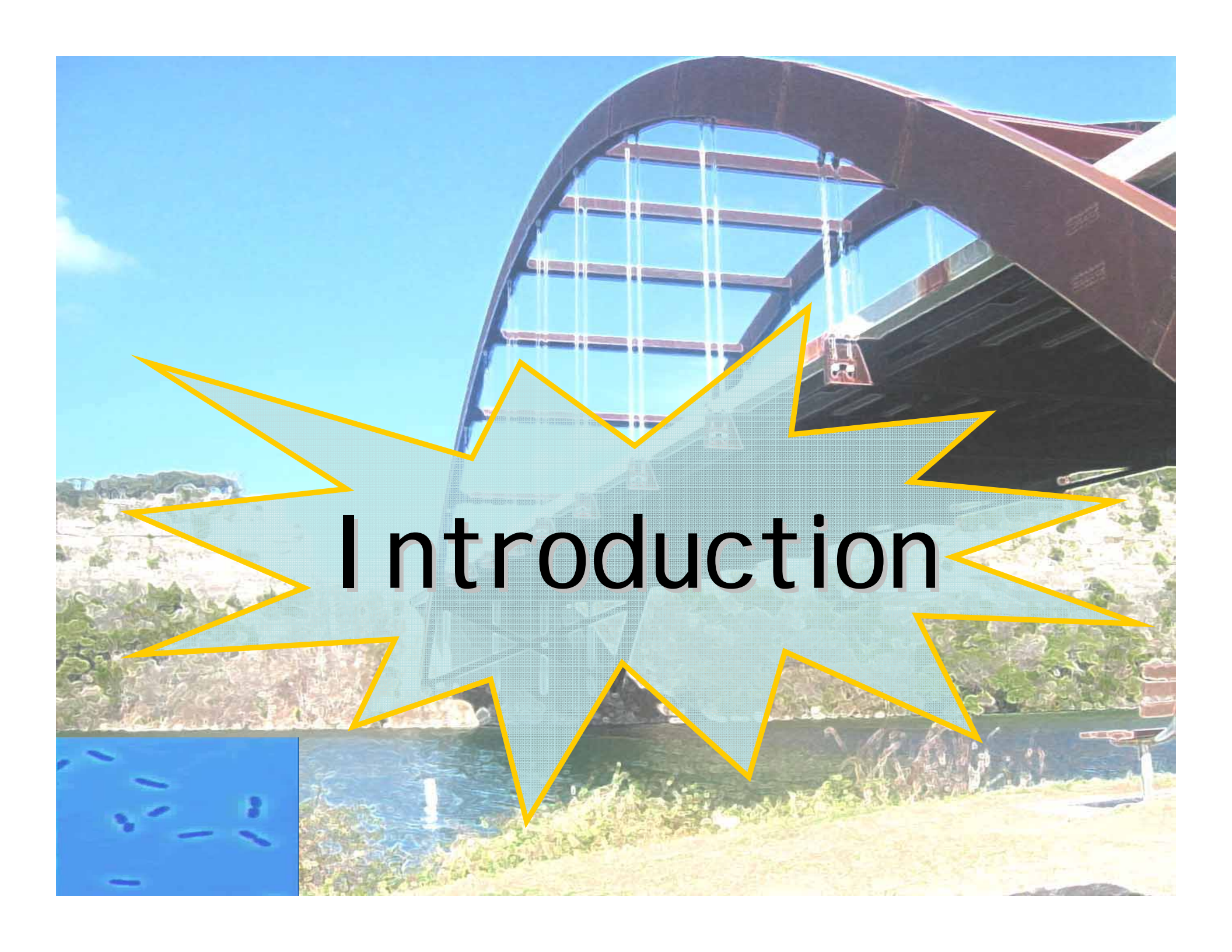
The University of Texas at Austin

- **Ruthie Elaine Luna**

Concentration: Microbiology

Standing: Senior

The University of Texas at Austin

The image is a composite. The background is a photograph of a suspension bridge with a large, curved arch, set against a clear blue sky. In the foreground, there is a body of water with a fountain spraying upwards. The bottom-left corner shows a microscopic view of several blue, rod-shaped bacteria. A large, yellow-outlined starburst graphic is centered over the image, containing the text 'Introduction'.

Introduction

Recreational Waters

- **Recreational Waters:**
 - body of water used for recreation (swimming, soaking, boating, etc.)
 - structure that encloses this water (CDC)
- **Types of Recreational Waters:**
 - treated or disinfected
 - untreated or naturally occurring

Examples of Recreational Waters

- **Treated or disinfected:**

- swimming pools
- water parks
- spas
- hot tubs

- **Untreated or naturally occurring:**

- streams, rivers
- creeks
- springs
- ponds, lakes
- beaches, oceans



http://www.mediapropictures.com/img/locations/lakes_rivers/bucegi%20lacul%20bolboci.jpg



<http://www.friendly-bungalows-tanna-vanuatu.com/images/freddy-on-beach.jpg>



<http://www.southtravels.com/america/usa/hyattregencyaustin/gifs/pool.jpg>



<http://www.hot-tub-direct.co.uk/newImages/sowers/spa1.jpg>

Recreational Water Illnesses

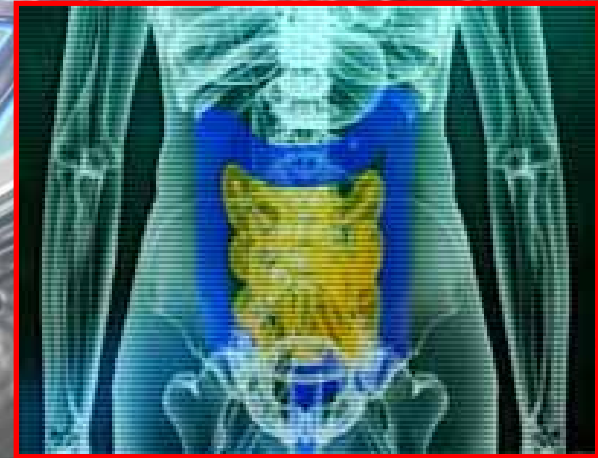
- **Recreational Water Illnesses (RWIs)**

- Any illness caused by exposure to contaminated water

- accidental defecation
- sewage
- wounds from diseased individuals

Manifestations of RWI s

- **Gastroenteritis (most common)**
- **Dermatitis**
- **Meningoencephalitis**
- **Folliculitis**
- Asymptomatic excretion
- Abdominal cramps
- Acute respiratory infections
- Hemorrhagic colitis
- Death



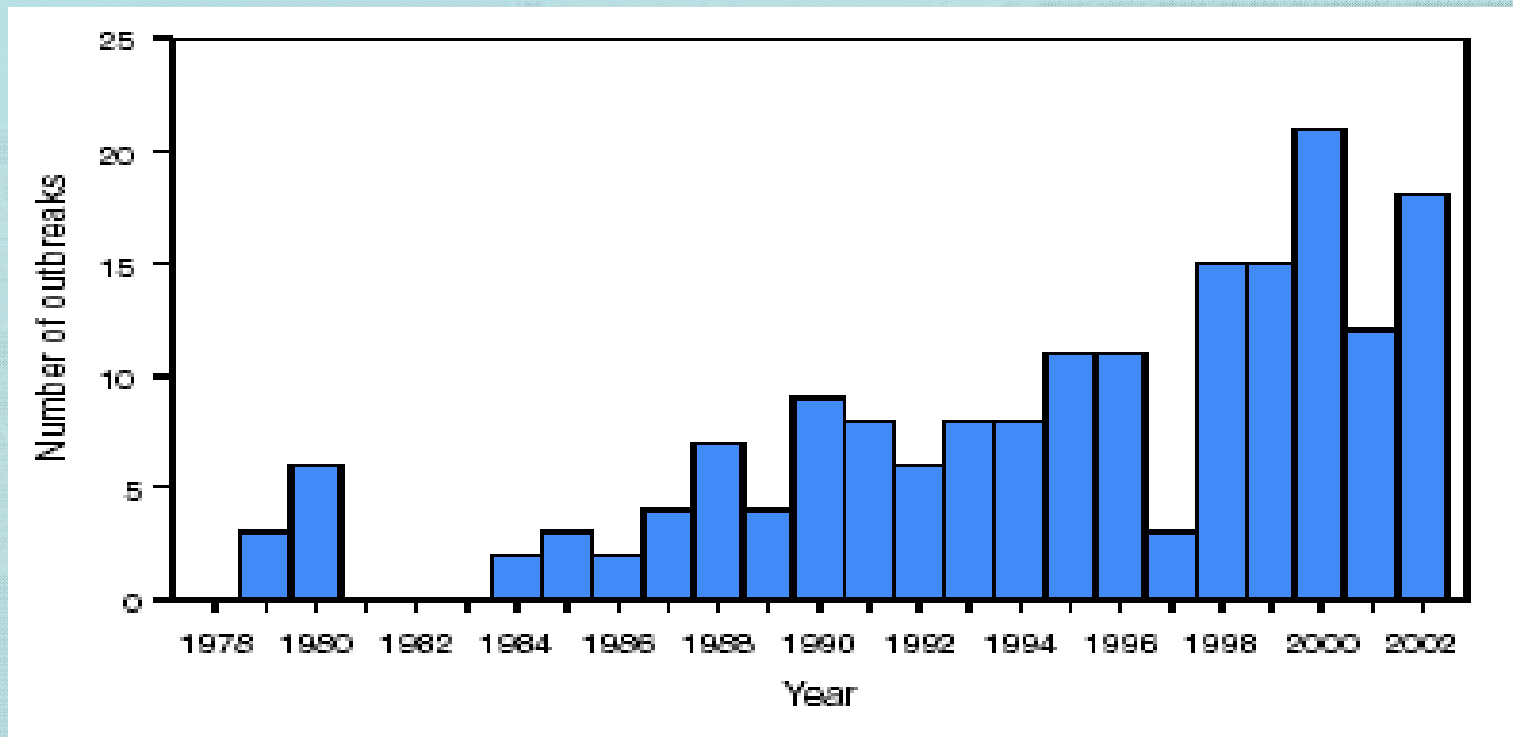
http://motrildigital.blogia.com/upload/2004-07-14_gastroenteritis.jpg



<http://www.dermatitisherpetiformis.org.uk/library/legs1.jpg>

Waterborne-Disease Outbreaks of Gastroenteritis Associated with Recreational Water

Number of waterborne-disease outbreaks of gastroenteritis (n=176) associated with recreational water, by year – United States, 1978-2002

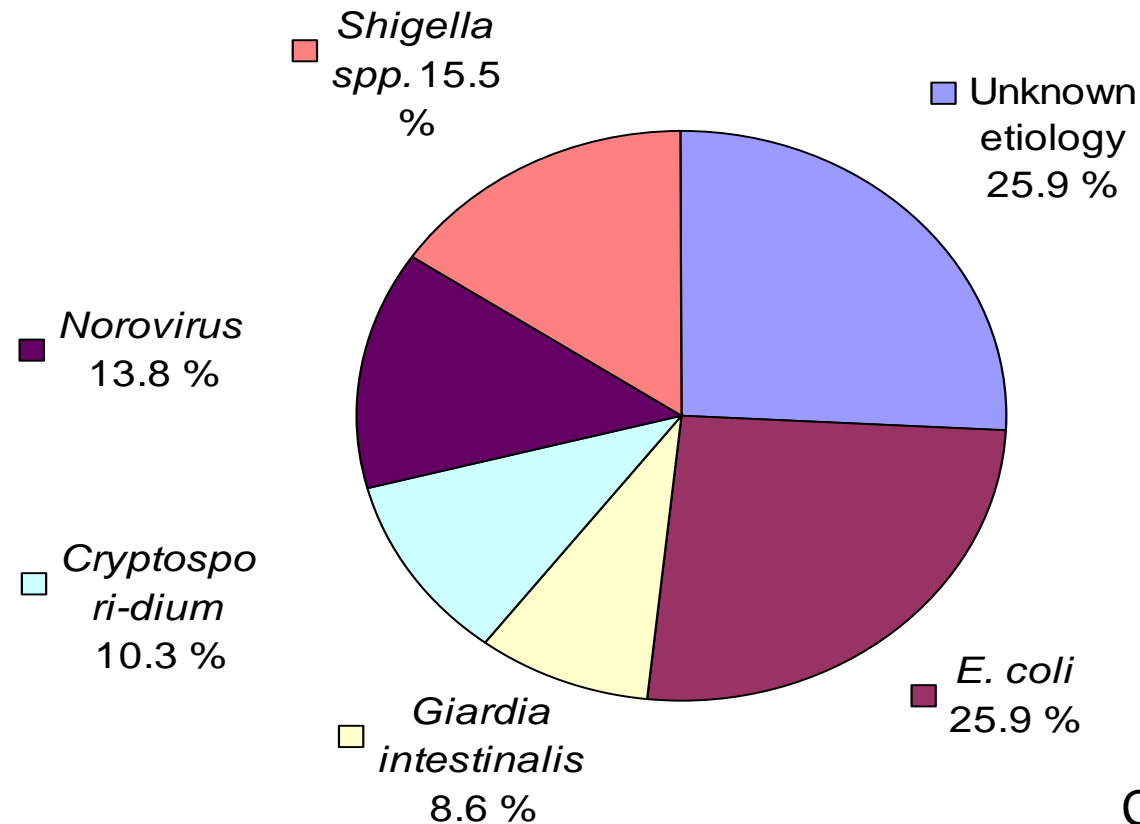


Facts and Figures

- **2001 to 2002: 65 outbreaks affected 2,536 people nationwide.**
- **Organisms found in ALL recreational waters associated with gastroenteritis:**
 - *Cryptosporidium* species (36.7%)
 - *Norovirus* (16.7%)
 - *Escherichia coli* (13.3%)
 - *Shigella sonnei* (6.7%)
 - *Giardia intestinalis* (3.3%)
 - Unknown etiology (AGI) (23.3%) (CDC)

Distribution of Etiological Agents in Untreated Recreational Waters Associated with Gastroenteritis

Etiological Agents in Fresh Water (n=58)



CDC

Monitoring Recreational Waters

1. Total Coliforms

- aerobic or facultative anaerobic
- Gram-negative
- non-spore-forming
- rod-shaped
- ferment lactose

2. Fecal Coliforms

- aid in the digestion of food
- grow at elevated temperatures
- associated with fecal material

Standards for the State of Texas

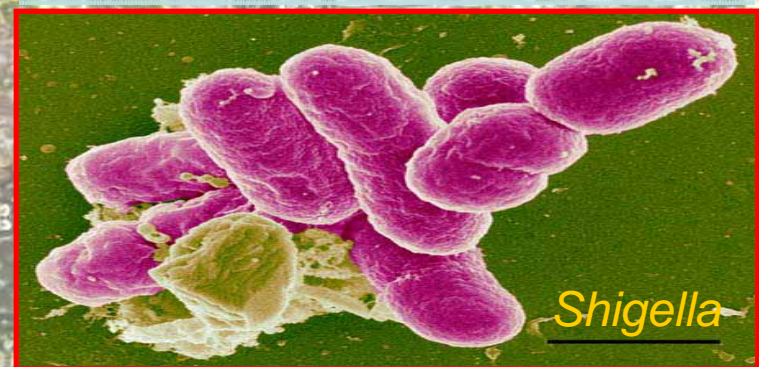
- No standard for the acceptable level of coliforms for fresh water spots.
- Regulation only occurs if a severe outbreak is reported.
- Early detection of harmful bacteria is beneficial.
 - outbreak prevention
 - public health

Coliforms are Members of the Family *Enterobacteriaceae*

- **Family:**
 - *Enterobacteriaceae*
 - **Genera:**
 - Frank pathogens
 - *Shigella*
 - *Salmonella*
 - *Yersinia*
 - *E. coli* O157:H7
 - Opportunists
 - *Enterobacter*
 - *Citrobacter*
 - *Escherichia*
 - *Klebsiella*



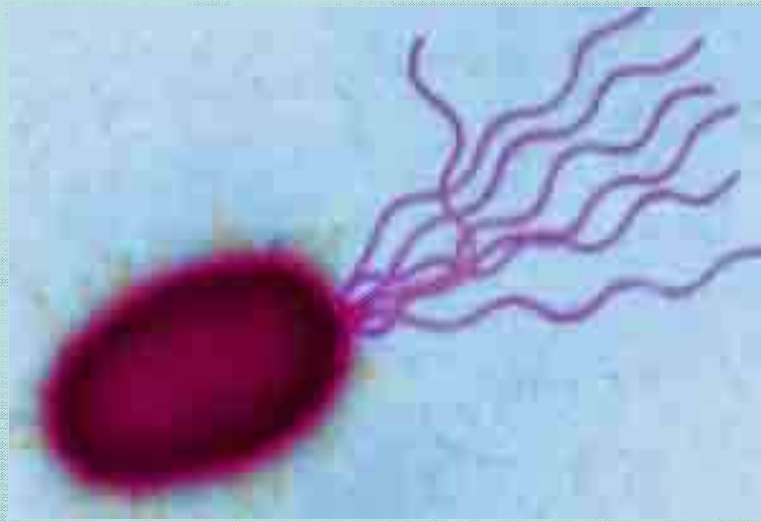
http://upload.wikimedia.org/wikipedia/commons/thumb/5/52/Citrobacter_freundii.jpg/280px-Citrobacter_freundii.jpg



<http://www.microscopyconsulting.com/Gallery/images/Shigella%20flexnarii.jpg>

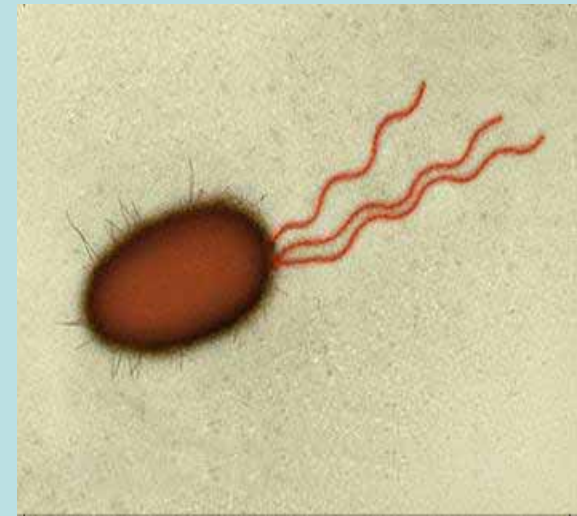
Escherichia coli

- Discovered by Dr. Theodore von Escherich in 1885.
- Gram negative bacteria that are usually found in the gut of healthy persons and other animals.
- Most are non-pathogenic to man; however, some strains can cause diarrhea and other diseases.

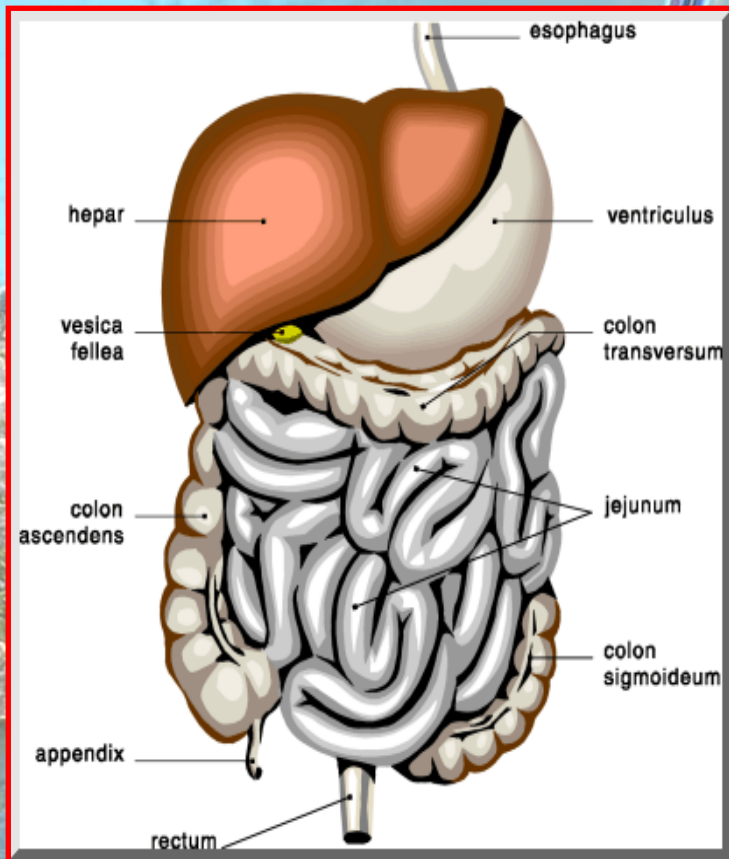


Escherichia coli O157: H7

- Emerging pathogen
- Affects 73,000 people and kills about 61 per year in the US.
- Transmitted by contaminated water, food, and recently recreational water.



Symptoms of Escherichia coli O157: H7 Disease



- Symptoms include:
 - Watery diarrhea
 - Bloody diarrhea
 - Abdominal pain
 - Hemorrhagic colitis

Complication:

- Hemolytic Uremic Syndrome (HUS)

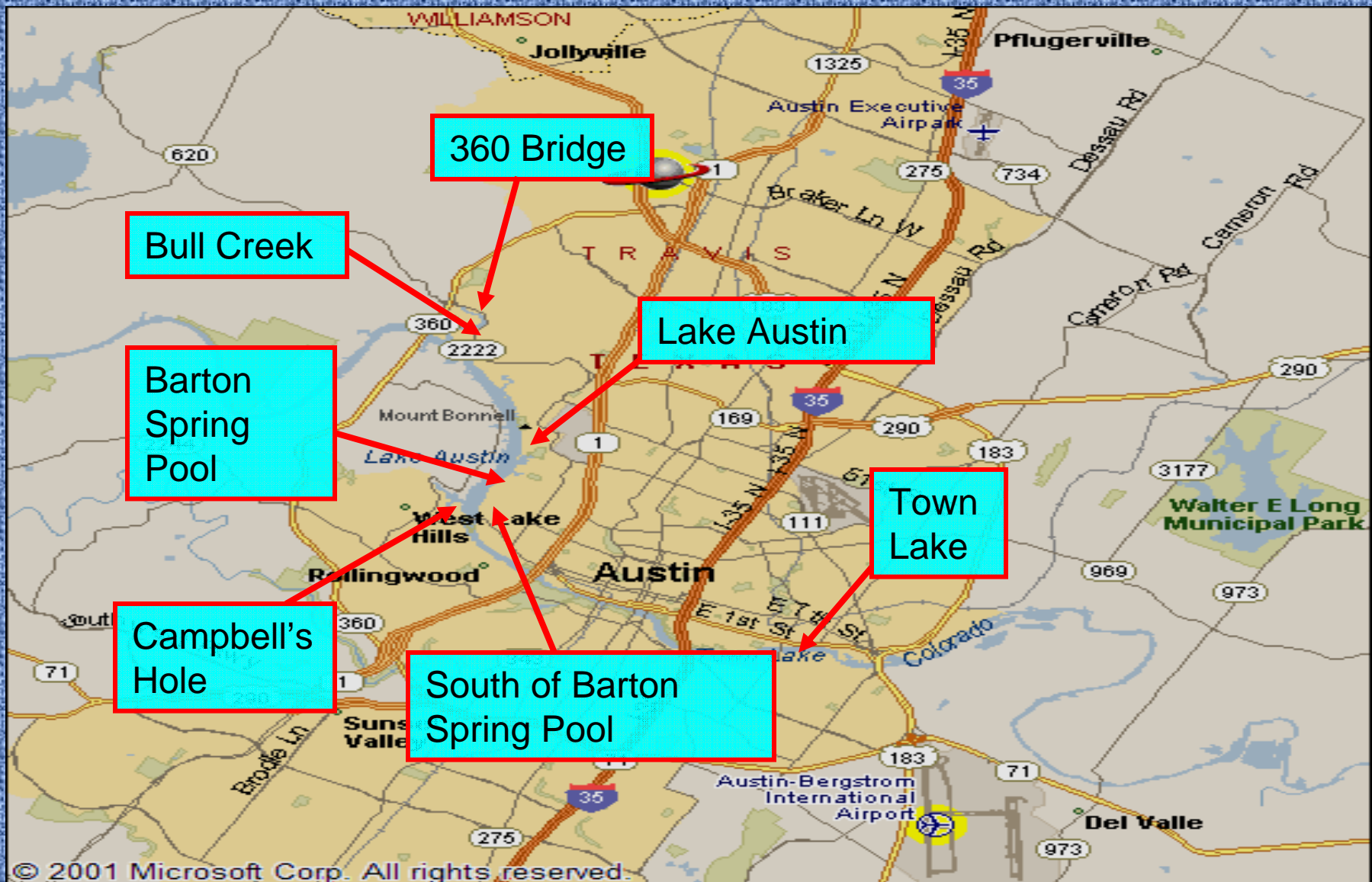
Purpose

Isolate and identify *Escherichia coli* 0157:H7 and other members of the family *Enterobacteriaceae* in recreational waters in Austin, TX and its surrounding areas.

A photograph of a park scene. In the foreground, a dark-colored dog is standing on a rocky shore next to a body of water. In the middle ground, a concrete dam with a metal fence runs across the frame. A person is visible near the dam. The background shows a grassy hill with trees and a utility pole. A large, light blue starburst graphic with a yellow outline is centered over the image, containing the word "Methods" in a bold, black, sans-serif font.

Methods

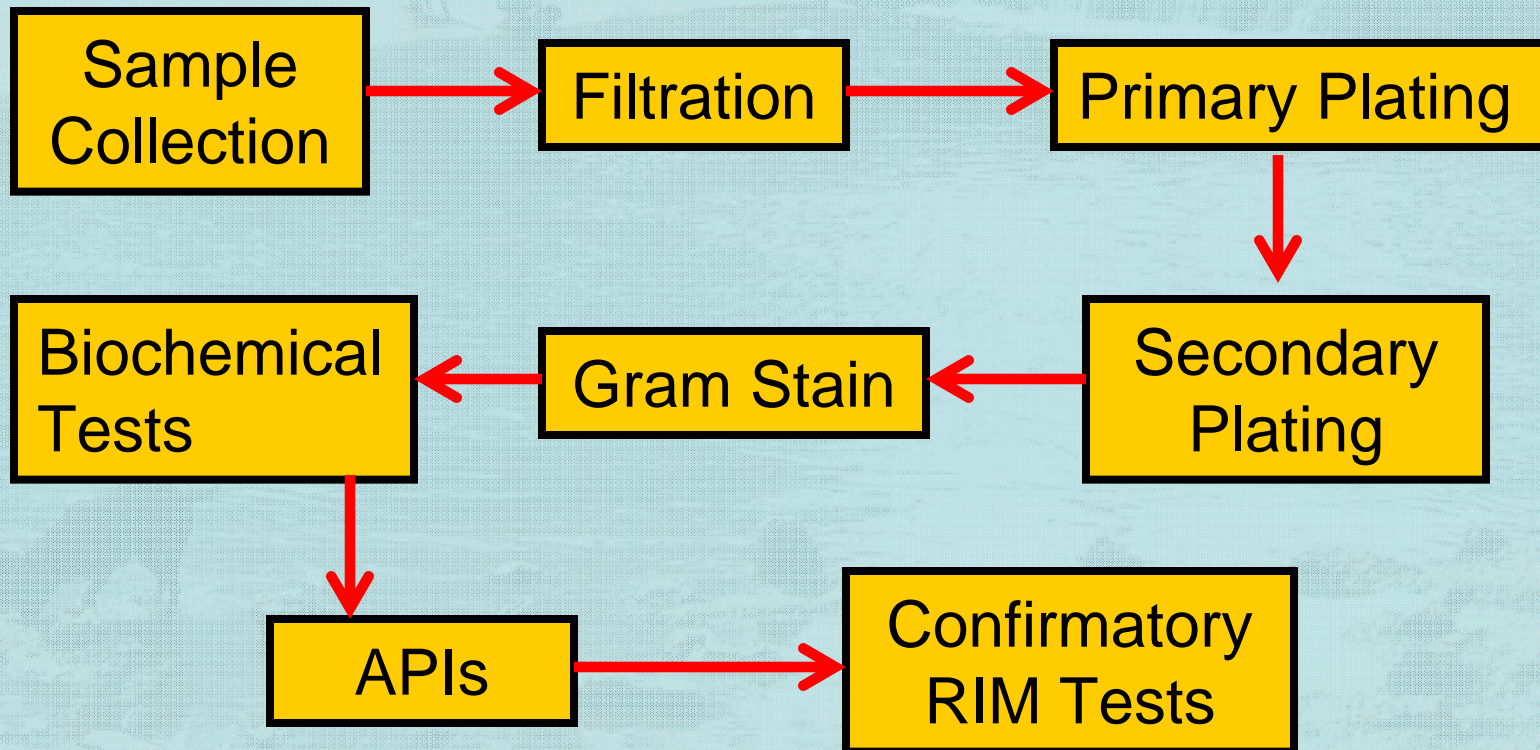
Recreational Water Sources



Study Design

- 7 sites
- Samples were collected weekly
- 188 water samples collected and analyzed

Protocol for Isolation and Identification of Members of the Family *Enterobacteriaceae*



Sample Collection

Samples were collected with sterile collection cups using standard protocol.



Transportation of Samples

Samples were transported to the Consumer Microbiology Laboratory at the TDSHS.



Sample Filtration

Samples were filtered using a membrane filtration system.



Primary Plating Media

MacConkey (MAC)



**Tellurite-Cefixime
Sorbitol MacConkey
(TCSMAC)**



Secondary Plating Media

MAC

Lactose +
(Pink)

Trypticase
Soy Agar
(TSA)

Lactose -
(Neutral)

Trypticase
Soy Agar
(TSA)

TCSMAC

Sorbitol +
(Pink)

Trypticase
Soy Agar
(TSA)

Sorbitol -
(Neutral)

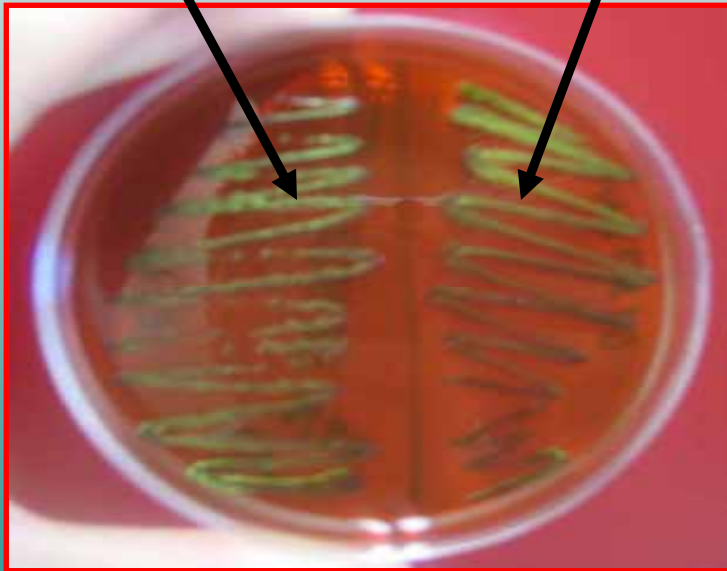
Eosin and
Methylene
Blue (EMB)

PSMUG Agar
Plate

EMB Agar Plate

E. coli 0157: H7

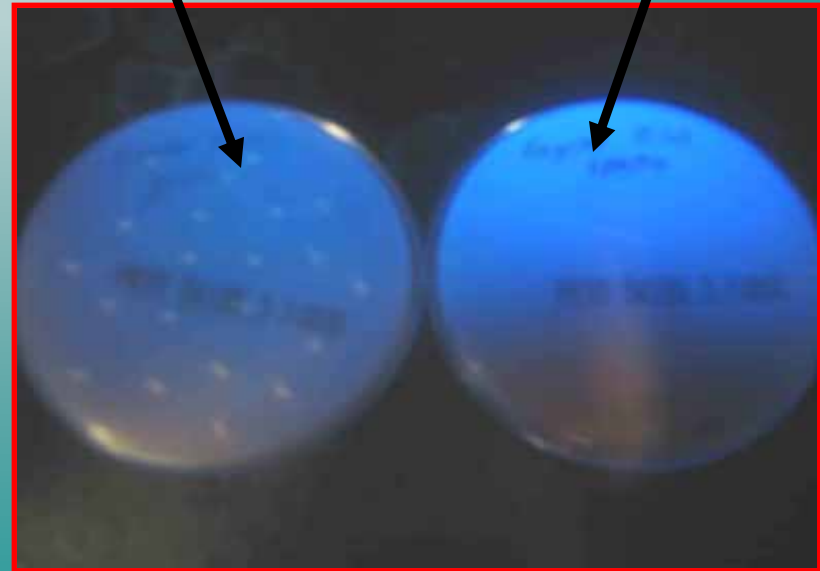
E. coli



PSMUG Agar Plate

E. coli

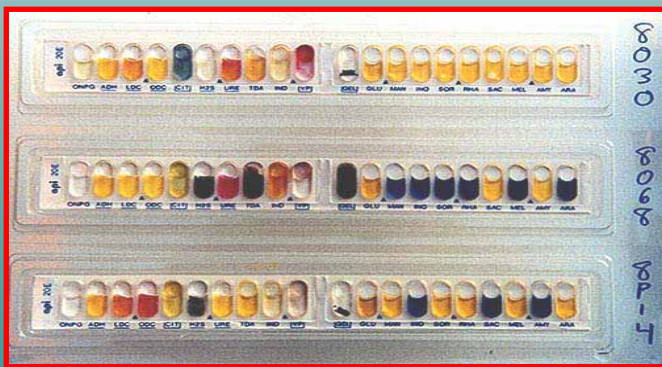
E. coli 0157: H7



Biochemical Tests



API-20E Biochemical Test System



<http://www.jlindquist.net/generalmicro/GBimages/API2.jpg>




Confirmatory RIM Test

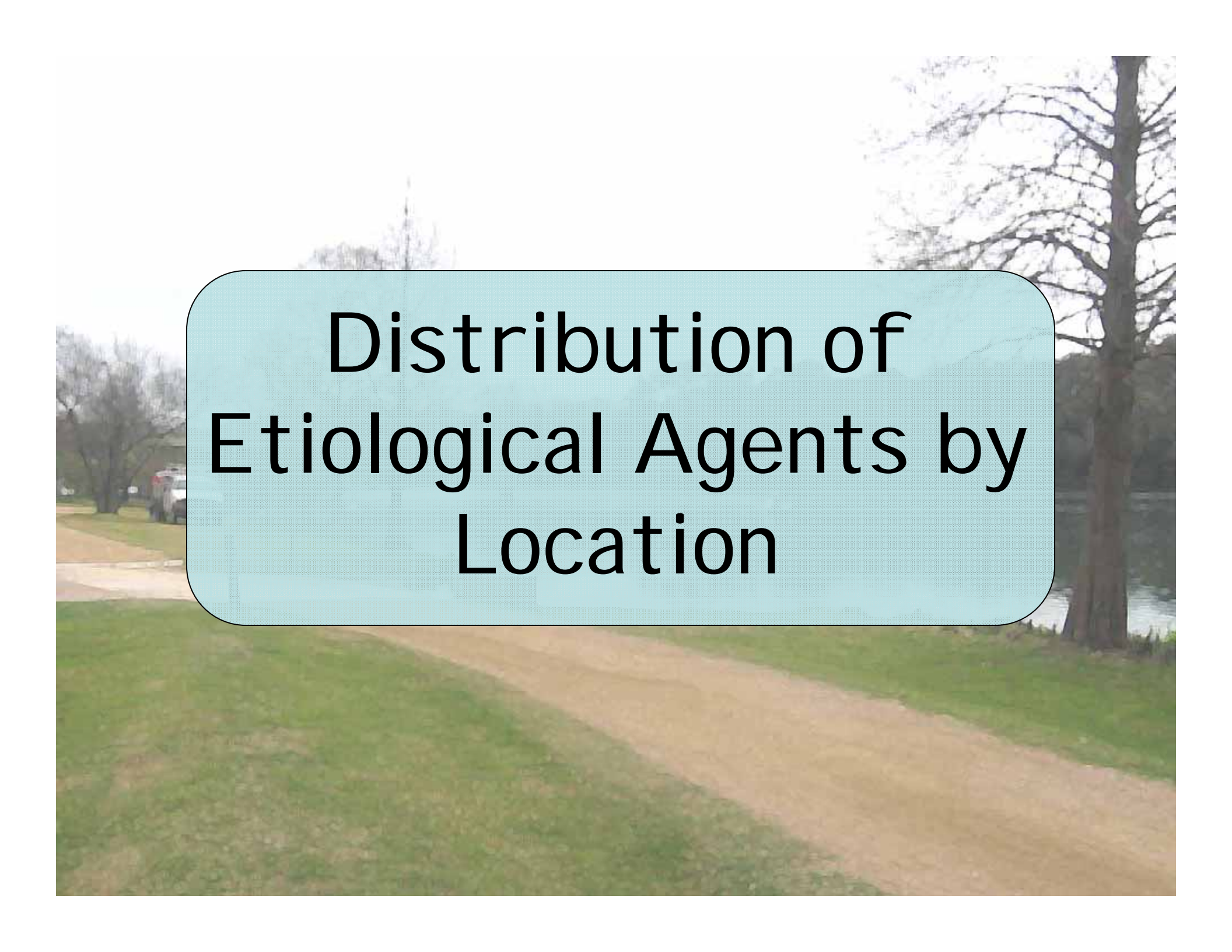


RIM





Results

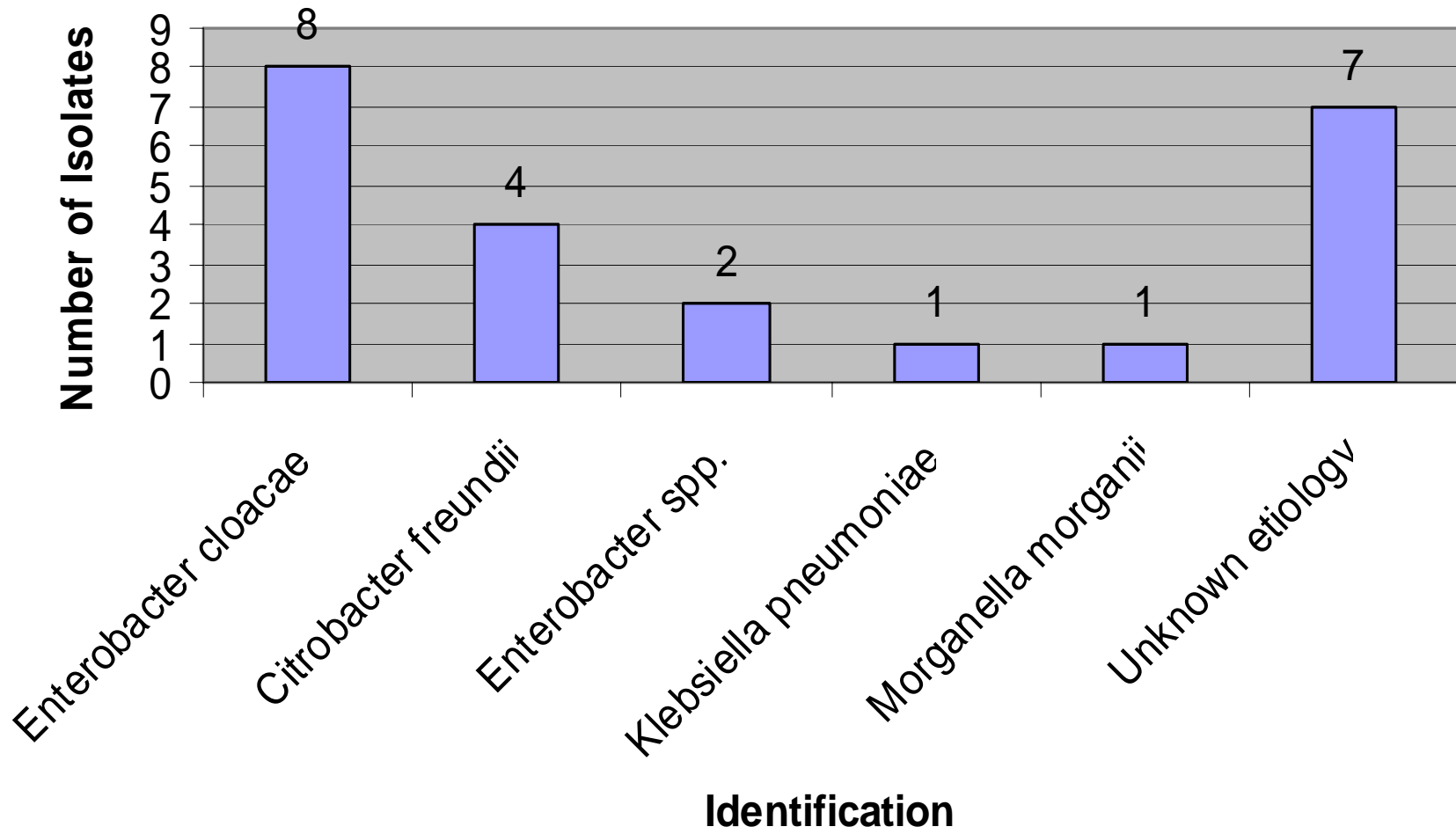
The background of the slide is a photograph of a golf course. A wide, light-brown dirt path or fairway runs diagonally from the bottom left towards the middle right. The surrounding area is covered in green grass. In the background, there are several trees, some with bare branches and some with green leaves. A white vehicle is partially visible on the left side. The sky is overcast and grey.

Distribution of Etiological Agents by Location

Pennybacker (360) Bridge



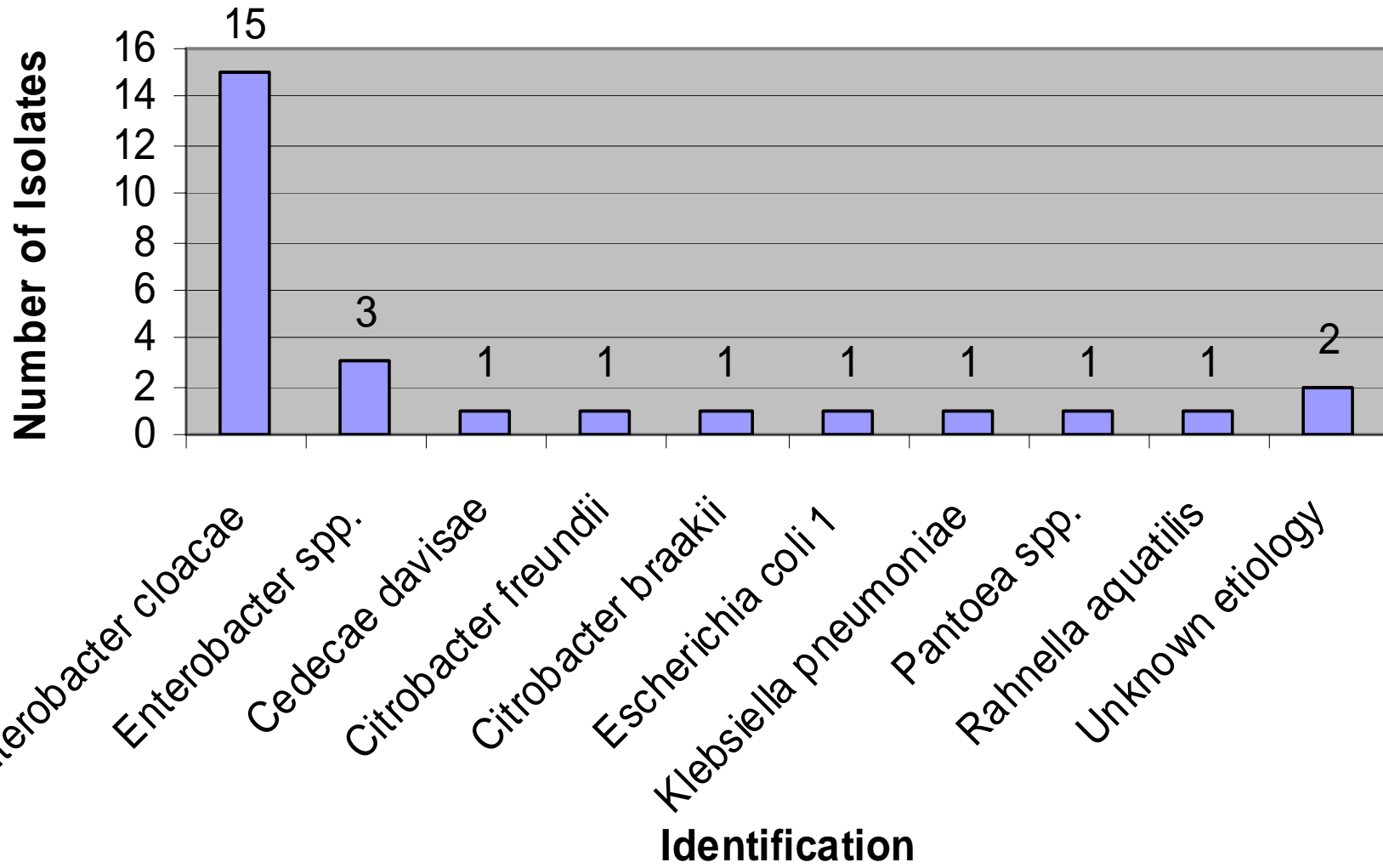
**Distribution of Members of the Family *Enterobacteriaceae* at
Pennybacker (360) Bridge
February - April, 2006 (n=23)**



Lake Austin



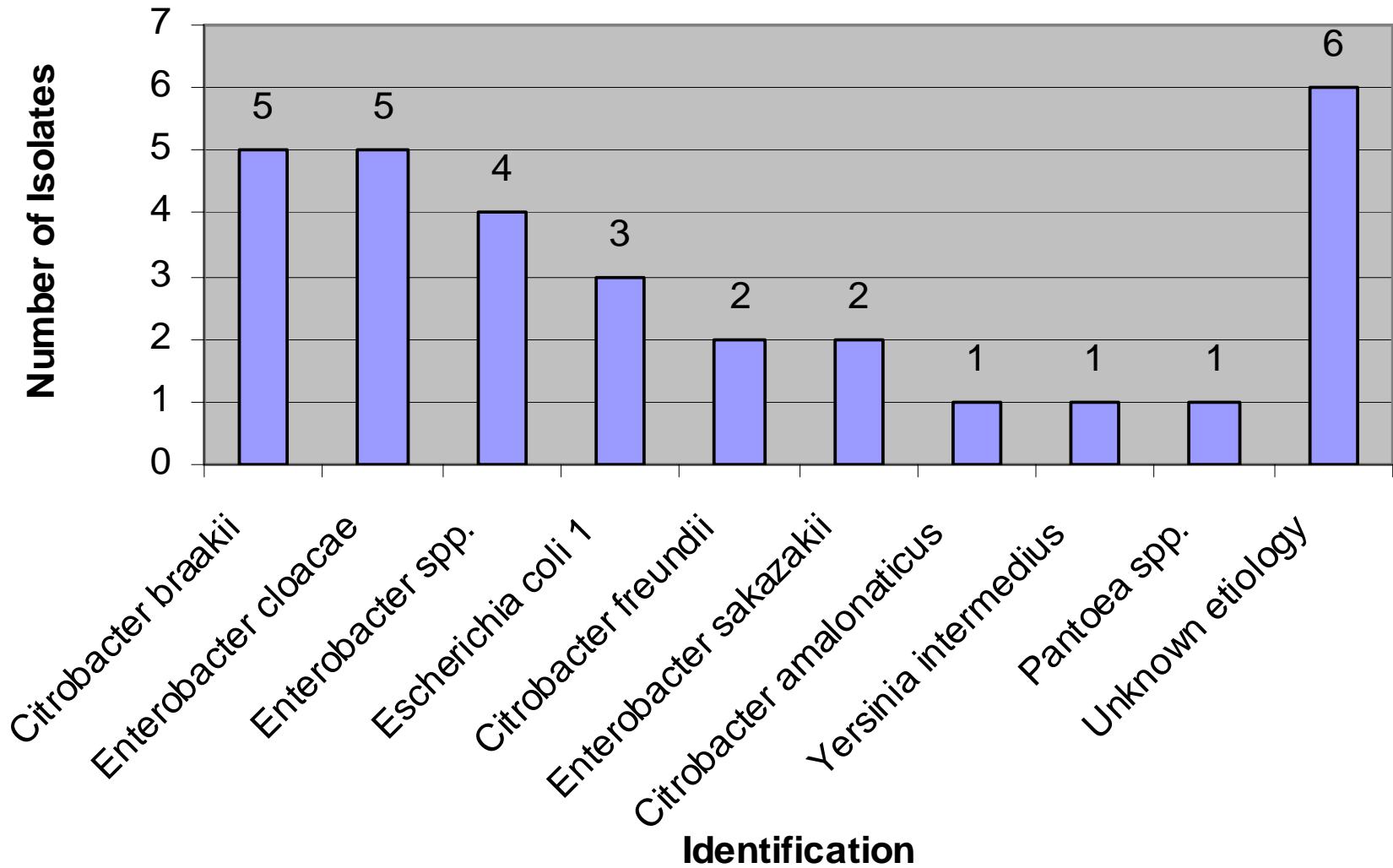
**Distribution of Members of the Family *Enterobacteriaceae* at Lake Austin
February - April, 2006 (n=27)**



Bull Creek



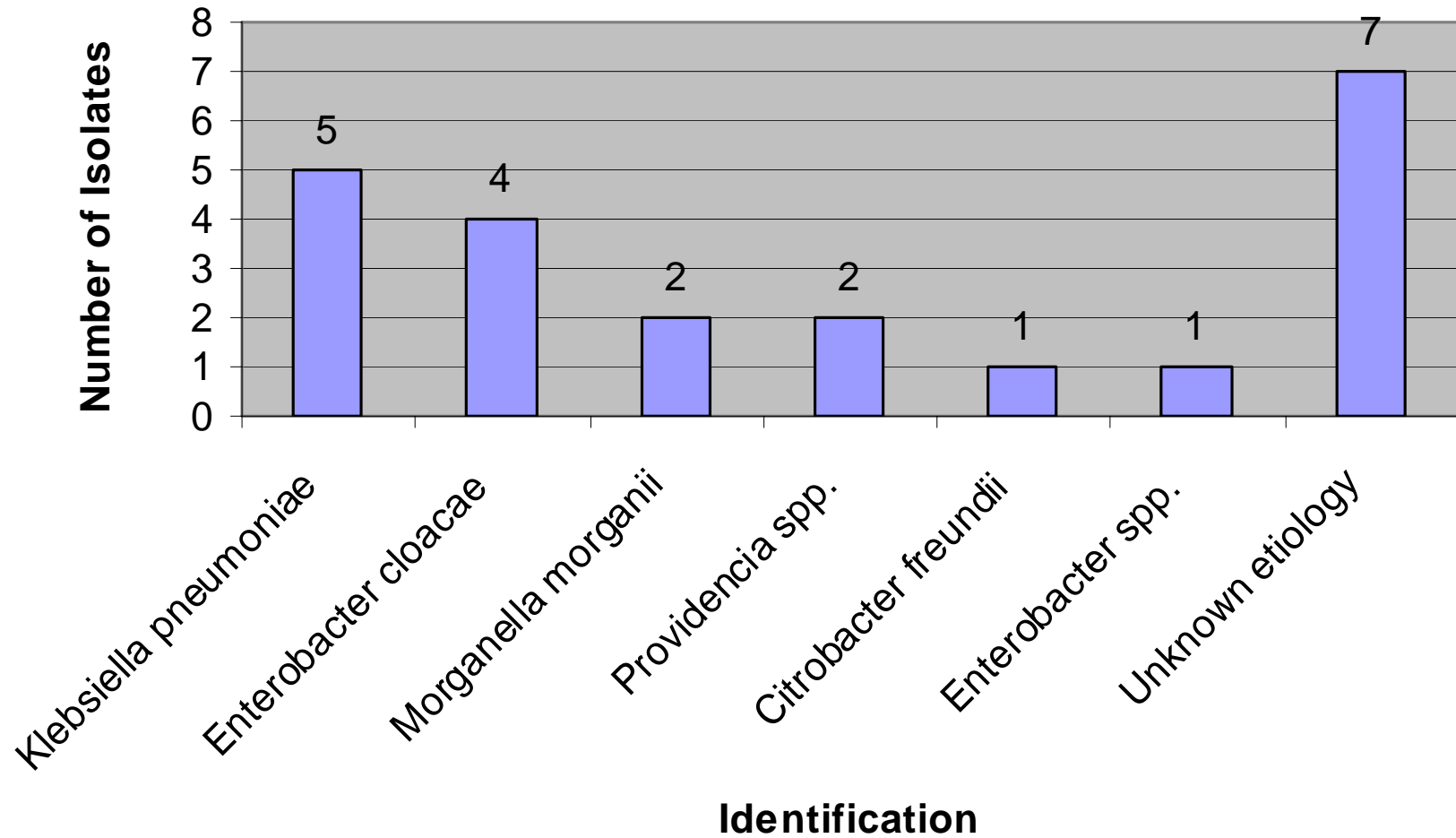
**Distribution of Members of the Family
Enterobacteriaceae at Bull Creek
February - April, 2006 (n=30)**



Barton Spring Pool



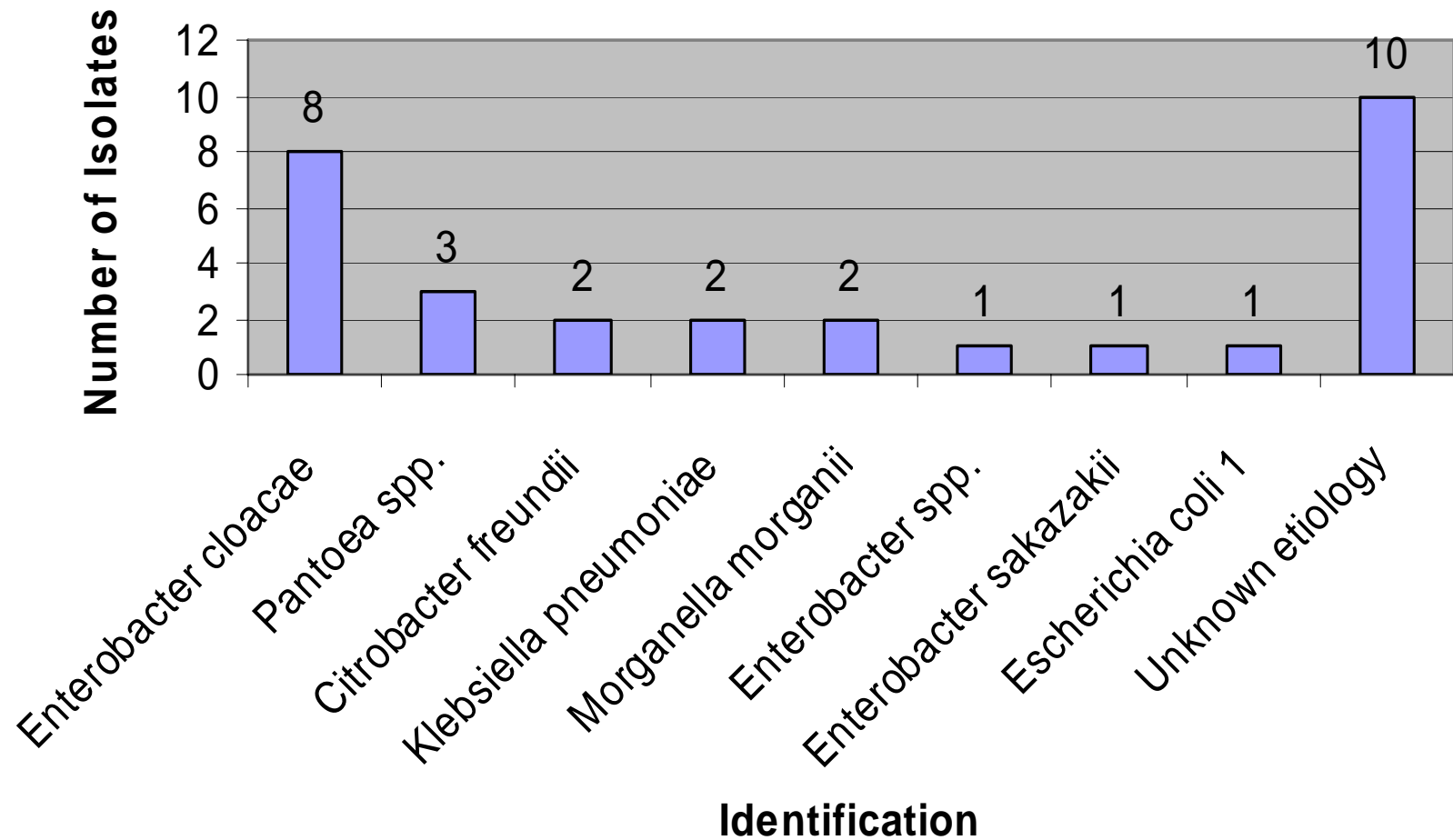
**Distribution of Members of the Family *Enterobacteriaceae* at
Barton Spring Pool
February - April, 2006 (n=22)**



South of Barton Spring Pool



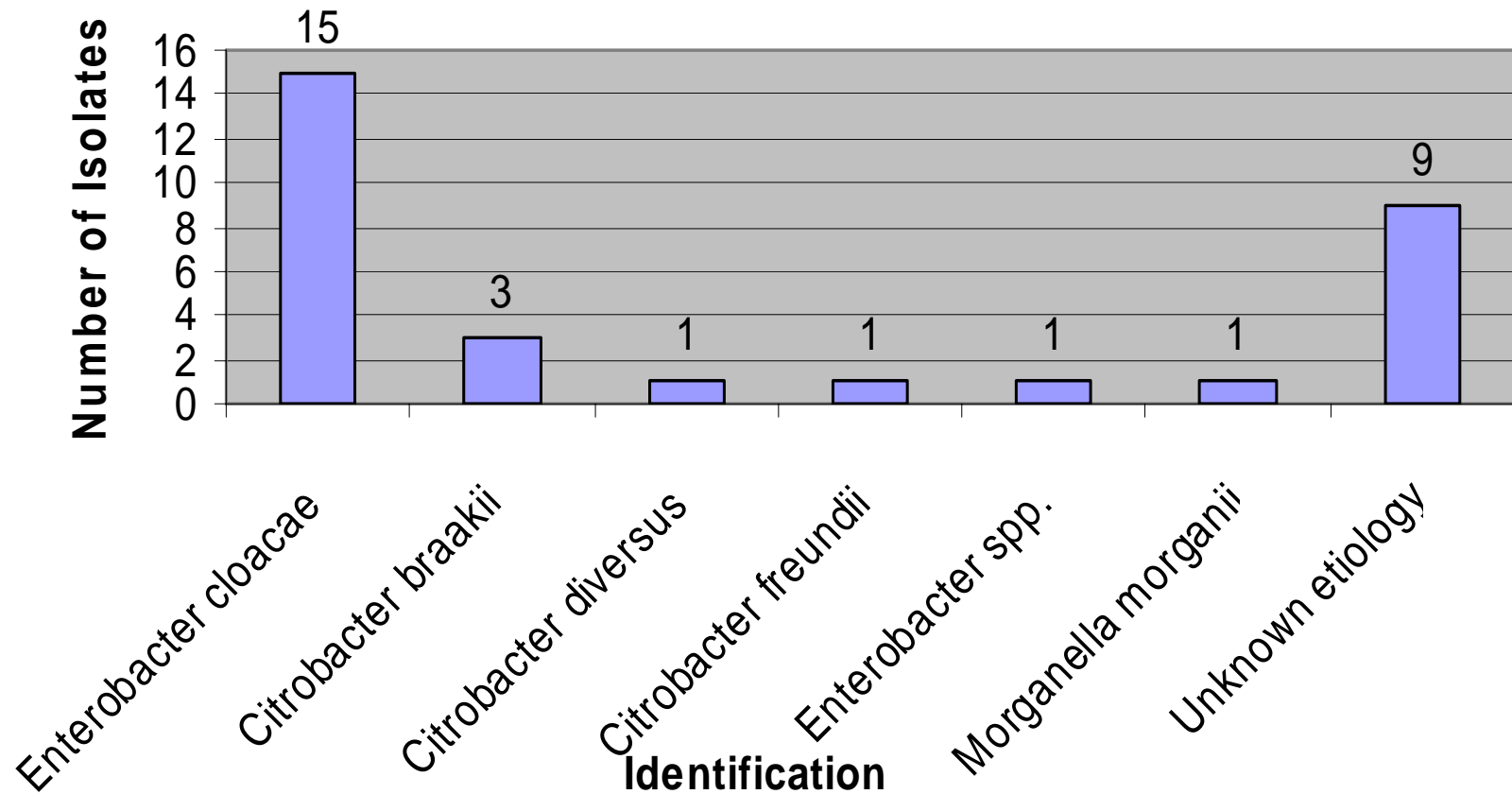
**Distribution of Members of the Family *Enterobacteriaceae* at
South Barton Spring
February - April, 2006 (n=30)**



Campbell's Hole



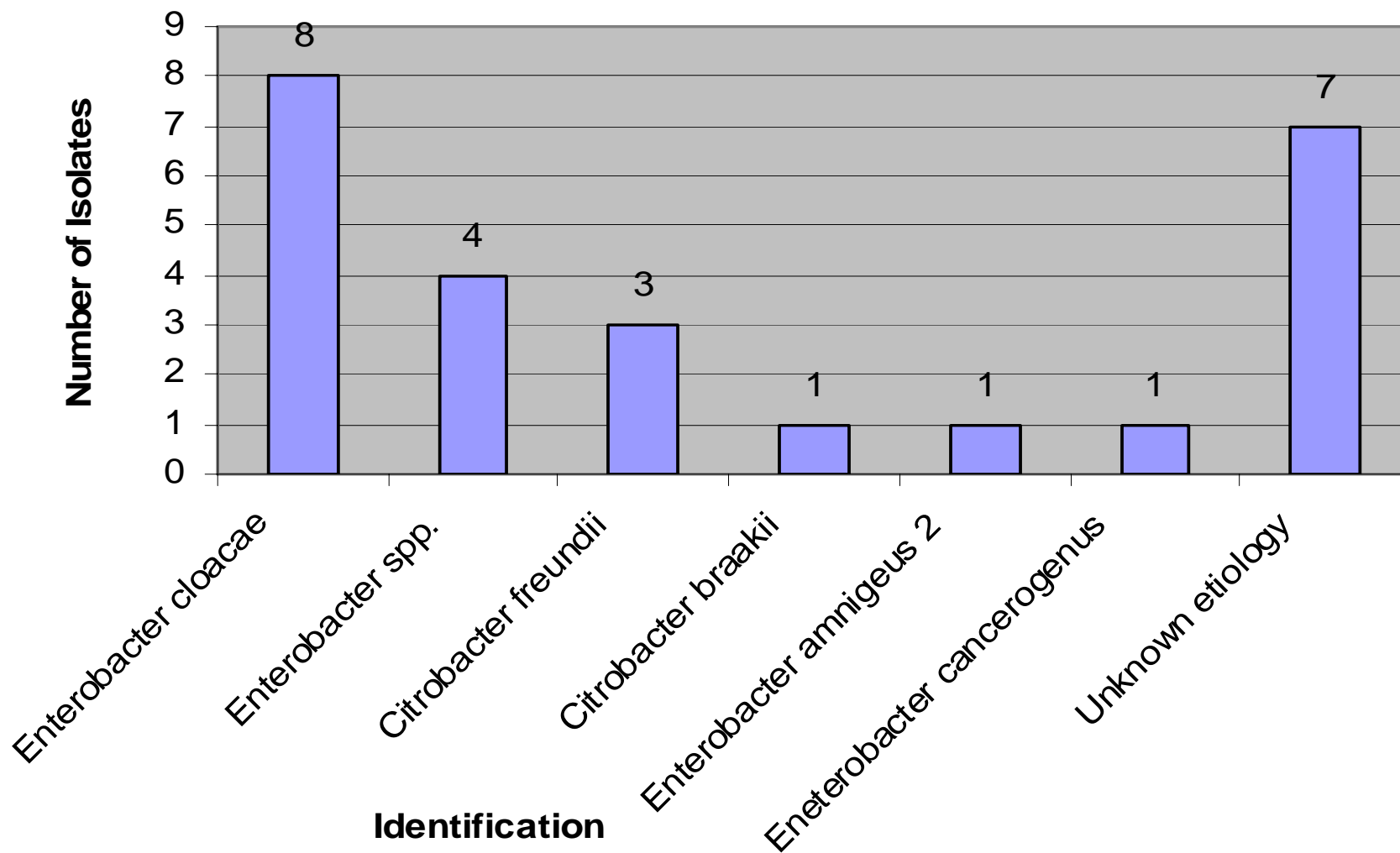
**Distribution of Members of the Family *Enterobacteriaceae* at
Campbell's Hole
February - April, 2006 (n=31)**

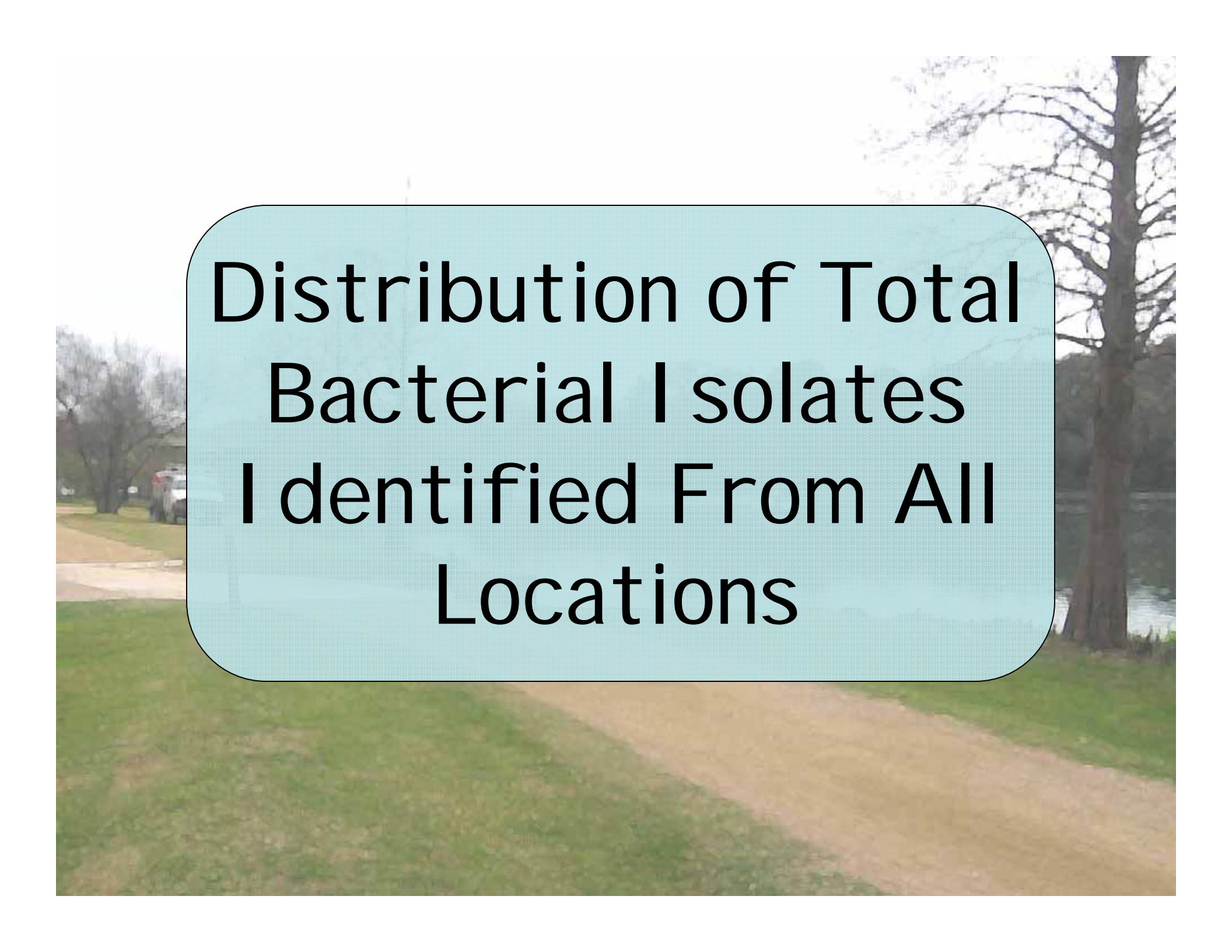


Town Lake



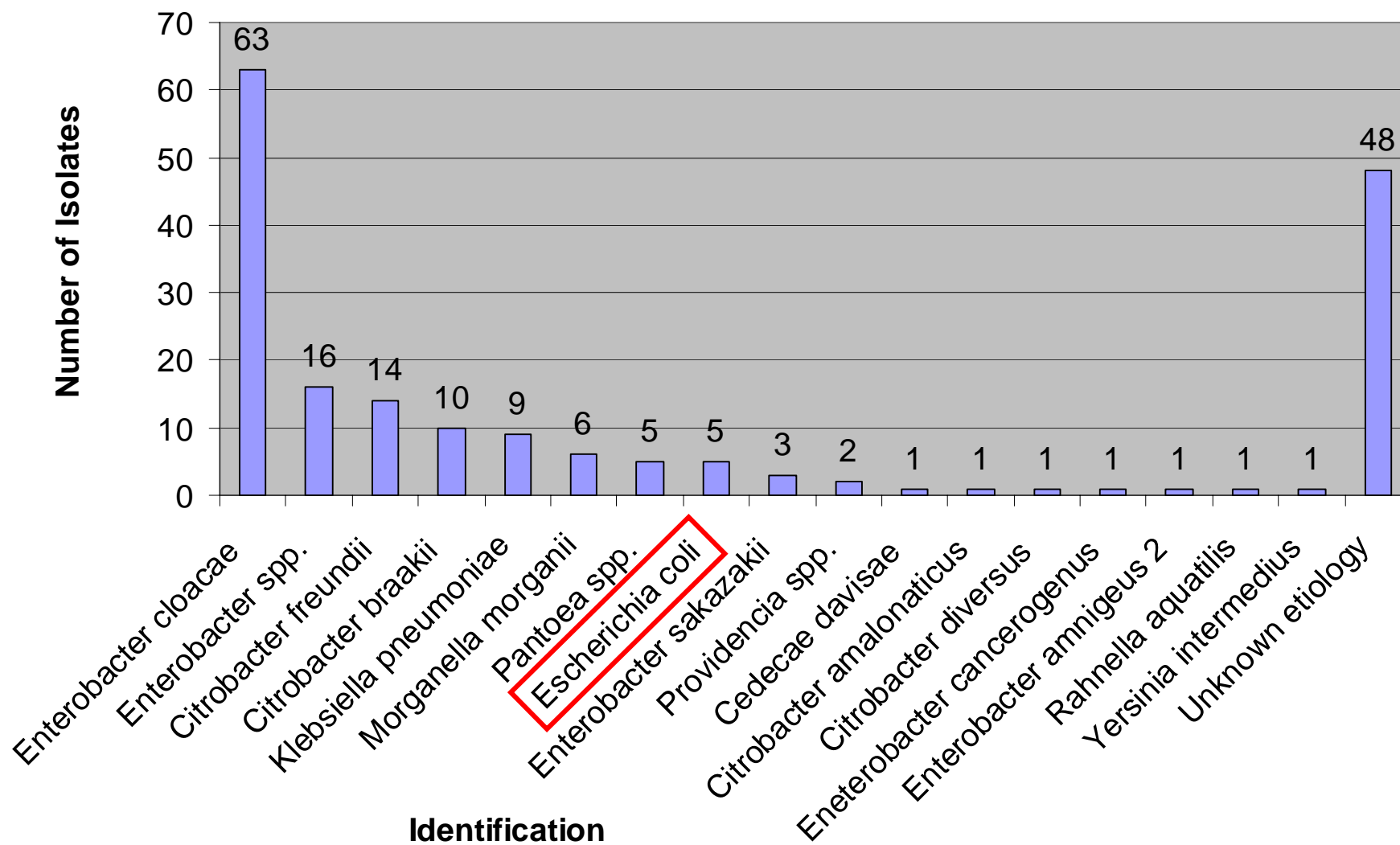
**Distribution of Members of the Family *Enterobacteriaceae* at
Town Lake
February - April, 2006 (n=25)**

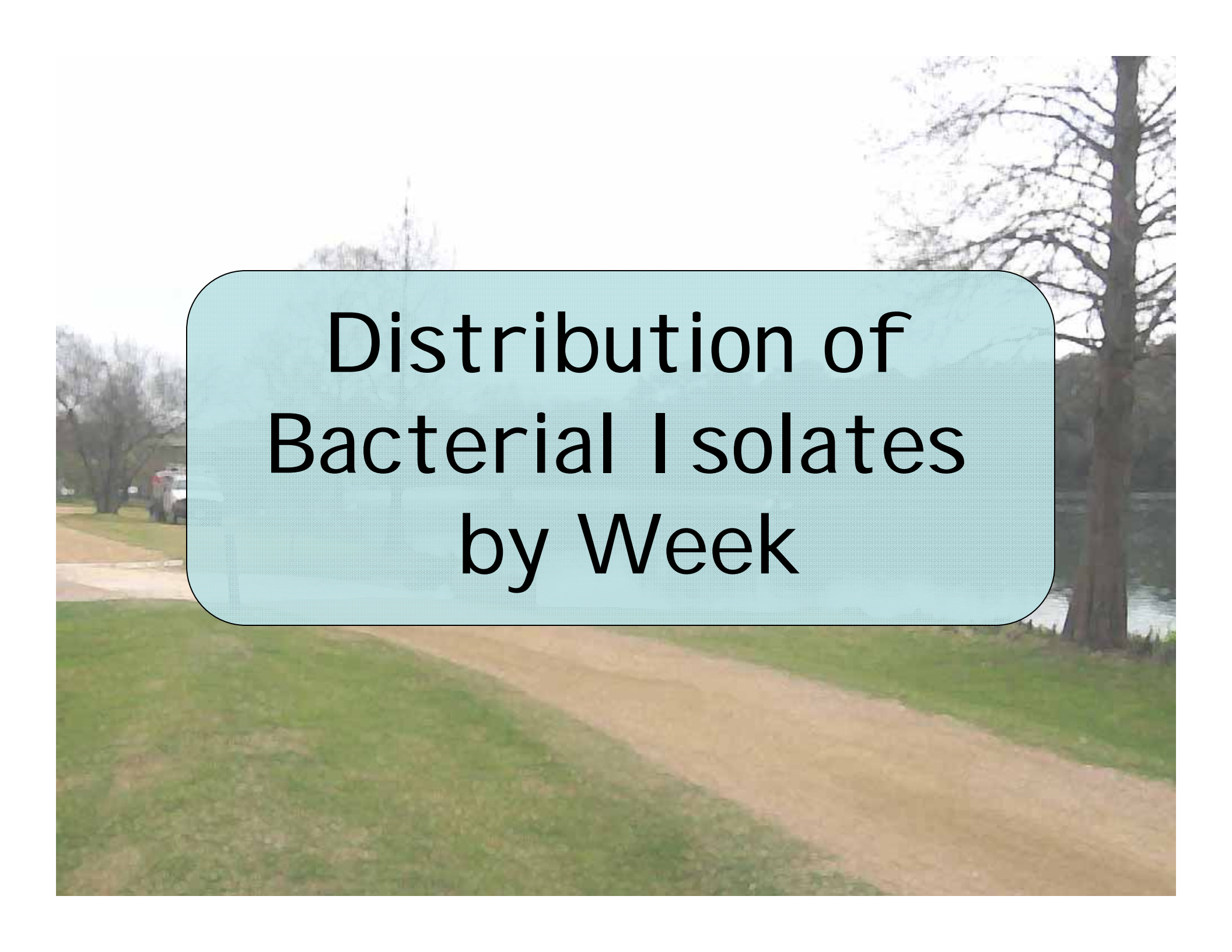


The background of the slide is a photograph of a golf course. A dirt path winds through a green lawn. In the distance, there are several trees, some with bare branches and some with light-colored blossoms. A white vehicle is partially visible on the left side of the path. The overall scene is bright and appears to be outdoors during the day.

Distribution of Total Bacterial Isolates Identified From All Locations

**Distribution of Members of the *Enterobacteriaceae* Family
Isolated from Recreational Waters in and Around Austin, TX
February - April, 2006 (n=188)**



The background of the slide is a photograph of a golf course. A light brown dirt path winds through a green lawn. In the distance, there are several trees, some with bare branches and some with green leaves. A white vehicle is partially visible on the left side of the path. The sky is overcast and grey.

Distribution of Bacterial Isolates by Week

Distribution of Total Etiological Agents Found Two or More Times over a 7 Weeks Period

	<i>E. cloacae</i>	<i>C. freundii</i>	<i>C. diversus</i>	<i>C. braakii</i>	<i>K. pneumoniae</i>	<i>M. morgani</i>	<i>P. spp.</i>
Town Lake	5	2	2				
Campbell's Hole	7		2				
Barton Springs	3						
South of Barton Springs	5	3					
Bull Creek	5	2		2	2	2	2
Pennybacker Bridge	3	2					
Lake Austin	7	2		2	2		
Total	35	11	4	4	4	2	2

A photograph of a paved walkway next to a body of water. The walkway is on the left, and the water is on the right. There are trees and a grassy area in the background. A large, light blue starburst graphic with a yellow outline is centered over the image, containing the word "Conclusion" in bold black text.

Conclusion

Conclusion

- **E. coli (5)**
 - Bull Creek (3)
 - Lake Austin (1)
 - South of Barton Spring (1)
 - None were O157:H7
- **No other frank pathogens recovered**

Conclusion (cont.)

- ***E. cloacae* – 63 (34%)**
 - Most frequent isolate
 - Consistently isolated over the 7 weeks period
- ***C. freundii* - 14 (7%)**
 - Second most frequent isolate
 - Less consistently isolated than *E. cloacae* from each site week to week
- **Unknown etiology - 48 (26%)**


Limitations

- Incubation at 37 C did not allow differentiation between coliforms and “fecal” coliforms.
- Difficult to determine significance of isolates (other than *E. coli*)
 - *C. freundii* and *E. cloacae*
 - Opportunistic human pathogens or may also be harmless environmental microorganisms.

The background image shows an outdoor swimming pool area. On the left, there is a body of water with a concrete edge. In the center, a white building with a flat roof is visible. To the right, a large, thick tree trunk extends from the top right corner towards the center. The sky is blue with some clouds.

Importance

- prevent public outbreaks of recreational water illnesses
- change the way public officials monitor and safeguard fresh water venues.



Future Studies

Future Studies

- Determine if coliforms present are of human origin by incubating isolates at 44°C.
- Repeat the study to quantify number of coliforms present.
- Longer surveys of recreational water locations.
- More locations around Austin, TX.
- Correlation between the number of etiological agents found from one location at different seasons of the year.

The background of the slide features a photograph of a boat on a body of water. The boat's hull is dark, and its windows are visible. The water is a deep blue, and the sky above is a lighter blue. In the foreground, there is a sandy beach with some green vegetation. A large, multi-pointed yellow starburst graphic is superimposed over the center of the image, containing the text.

Acknowledgments

My heartfelt thanks to:

- **Ms. Monica Kingsley**
- **Ms. Miriam Johnson**



■ *Consumer Microbiology Team*

■ Crystal

■ Terry

■ Chris

■ Natalie

■ Tom

■ Maile



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Control and
Prevention**

**Epidemiology and Laboratory Capacity
for Infectious Diseases Program**

Thank You

**Dr. Leanne
Field**