Development of Microbiology Laboratory, An Antibiotic Stewardship Program, and Infectious Diseases Training in Republic of Georgia

Trip Report (May 2023): USAID Project on April 18 to May 2, Zugdidi, Georgia

Dr. Aliyar Cyrus Fouladkhah, PhD, MPH, CFS, CPH
Founding Director, Public Health Microbiology Foundation[SM]
Associate Professor, Tennessee State University
Yale School of Public Health Alumnus

Public Health Microbiology Foundation[SM] has donated supplies, media, and consumables for establishing microbiological procedures in the host institution in Zugdidi. Thanks to these suppliers, during this assignment, laboratory procedures were established for conducting microbiological analysis of veterinary clinic samples as well as various foods and water samples. One individual with a doctorate and the other, a doctoral candidate, were extensively trained for the conduct of these microbial analyses.

Additionally, a comprehensive antibiotic stewardship program was established for rapid selection (within few hours) of the most potentially efficacious antibiotic (on Gram-staining-guided bases) and antibiotic susceptibility test withing 24 to 48 hours. Several microbial samples including water and meat products obtained from the local market were analyzed by trainees of this assignment. The entrepreneur (Association for Agricultural Development in Zugdidi, Georgia) received extensive training about infectious diseases in their region, examination of various bacterial and mammalian tissues (selection of 100 microscope slides were donated from the Public Health Microbiology Foundation[SM]), microbiological analyses, and antibiotic stewardship. Extensive training was also conducted in biosafety to ensure the trainees and the host institution could ensure the safety of the lab members, the stakeholders, and the community.

This assignment was approximately 80% hands-on training and consultation in laboratory and 20% lecture-based traditional training. Excerpts of teaching material are provided below.

In summary these trainings were provided:

- Food Microbiology Analysis
- Water Safety Analysis
- Mammalian Tissue and Microbiology Examination Under Microscope
- Antibiotic Stewardship (Gram-staining Guided & Antibiotics Susceptibility Test)
- Infectious Diseases of Prevalent in Georgia and Zoonotic Diseases
- Biosafety Training for Microbiology Laboratory

The value of supplies provided by the Public Health Microbiology Foundation[SM] for this program is conservatively estimated at $2,500 USD, which enabled the host to established microbiological and antibiotic stewardship programs.

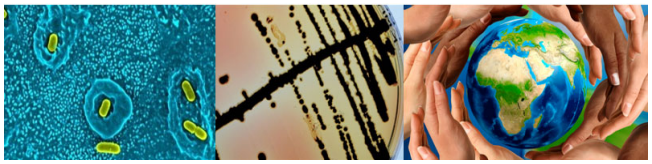
The microbiology training provided was on basis of U.S. FDA's Bacteriological Analytical Method and prior to commercial testing, the host is requested to obtain appropriate and specific testing standard for each microbiological analysis.

Special thanks are needed for great colleagues both in Washington and Tbilisi for all they have done to support this productive and impactful program.

Submitted with best wishes,

Aliyar Cyrus Fouladkhah, PhD, MS, MPH, MACE, CFS, CPH
Founding Director, Public Health Microbiology Foundation[SM]
Faculty Director, Public Health Microbiology Laboratory
Associate Professor, Tennessee State University
Yale School of Public Health Alumnus

Microbial Food Safety and Infectious Diseases Training
April 28, 2023, Zugdidi Georgia
Lead Instructor: Dr. Aliyar Cyrus Fouladkhah



Public Health Microbiology: Implication for Georgian Stakeholders

USAID Project, Zudgigi, Georgia

4-27-2023

Tennessee State University, Nashville, TN

A. Fouladkhah: Faculty Director, Public Health Microbiology Laboratory

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- Animal Science (Microbiology and Food Safety), PhD (CSU)
- Applied Statistics and Data Analysis, Graduate Certificate (CSU Statistics Dept.)
- Food Science & Human Nutrition, MS (CSU Food Science Dept.)

Yale SCHOOL OF PUBLIC HEALTH

- Biostatistics and Epidemiology, Advanced Professional MPH
- Food and Drug Regulatory Affairs, Graduate Certificate
- Climate Change and Health, Graduate Certificate

CPH Certified in Public Health

SAS Certified Base Programmer for SAS 9

Advanced Professional MPH Program

Website: https://ysph.yale.edu/school-of-public-health/graduate-programs/accelerated-mph-program/
Video: https://www.youtube.com/watch?v=IGVN9JfoIt8

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Public Health Microbiology Laboratory
Tennessee State University

MPH Curriculum Food Safety and Applied Epidemiology (now under CEPH certification)

- Secured extramural support >$4.5M as PD or Co-PD since 2015
- **Funding sources**

(1) Dean's Office: $10,000/year and a Research Technician

(2) Association of Food and Drug Officials (AFDO) Process Authority: $15-50K per year depending on the projects

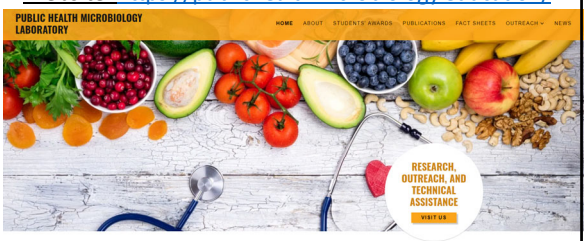
(3) Extramural Funding: >$4.5M since 2015

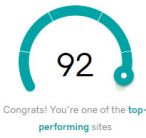
- National Institute of Health: $**33,680** (PD of Sub-award, 2020-21)*
- Pressure BioScience Inc.: **$35,000** (Role: PD, 2019-2024)
- USDA-NIFA CBG: **$350,000** (Role: PD, 2018-2022)
- USDA-NIFA HEC: **$50,000** (Role: PD, 2018-2021)
- USDA-NIFA FSOP: **$165,000** (Role: PD, 2018-2021)
- Pressure BioScience Inc.: **$23,500** (Role: PD, 2017-2019)
- USDA-NIFA FSOP: **$59,750** (Role: PD, 2016-2019)
- Pressure BioScience Inc.: **$9,400** (Role: PD, 2017-2019)
- NIFA FSOP.: **$880,000** (Role: CO-PD, 2019-2023)**
- USDA-NIFA FSOP.: **$1,197,751** (Role: CO-PD, 2015-2020)**
- NIFA CBG.: **$300,000** (Role: CO-PD, 2018-2022)

*Pending account setting and internal administrative approval.
** Sub-awardee of Southern Center Main Awards.

Public Health Microbiology Foundation
Compassion Service Excellence
Public Health Microbiology™ Foundation
Dr. Aliyar Cyrus Fouladkhah

Website: https://publichealthmicrobiology.education/





Website performance: 4/22/2020

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Teaching in Tennessee and Internationally

Additional Global Health Information Available at:
https://publichealthmicrobiology.education/global-health-programs

2022: Georgia, Columbia
March 20200/July 2021

2021, 2022 Jamaica
Nov./March 2021

2020, and 2022, Haiti
Haiti Government, Fortification with iron, vitamin b12, and zinc

2019, Philippi Township, Cape Town, South Africa:
HIV Prevention Training

2018, 2020, 2022 Guatemala
Food Safety Training for Food Industry Leadership

2017 Santiago, Dominican Republic
USAID Public Health and Microbiology Training Faculty and Staff of ISA University

Graduate Course in Food Policy and Regulations

2020 Student Evaluation:

- *"…Dr. Fouladkhah is easily the nicest professor I have ever had the pleasure of meeting. He seriously cares about you and how you're doing."*
- *""I loved this class it was so interactive and different from any other class I have taken here at TSU!"*

2019 Student Evaluation:

- *"Dr. Fouladkhah is an excellent professor. He does the absolute best job of making students feel comfortable making discussion in class and is exceptionally knowledgeable in the area of food sciences. The in class exercises are definitely helpful to make sure the lectures are being retained and assists in requiring little to no studying outside of the class meetings."*
- *"This course is top notch, one of the best courses I have ever taken, Much gratitude to the lead instructor Dr. Fouladkhah. I learned so much in the class and my knowledge on food policies and regulation has increased a thousandfold."*
- *"Everything was well organised, I think it is perfect. Nothing else is needed."*

2018 Student Evaluation:

- *"This man is so amazing. Learned so much in his class thank you Dr. Fouladkhah."*
- *"He is very helpful and always very encouraging. He helped me planned my studies and even future goals."*

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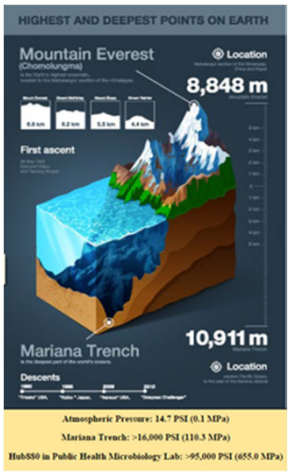
Research Responsibility:
1. Elevated Hydrostatic Pressure
2. Bacterial Biofilm
3. Effects of Climate Change on Infectious Diseases

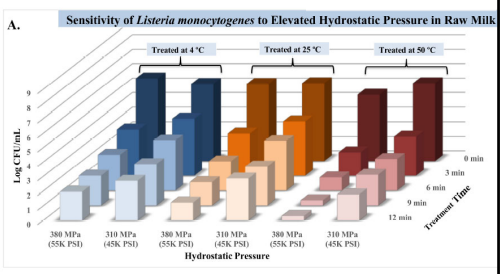
PBI Pressure BioSciences Inc.

- Elevated Hydrostatics Pressure:
- Hub880, up to **650 MPa**
- Deepest part of Oceans (Mariana Trench): c. **110 MPa**
- Programable unit Hub 440, 380 MPa
- Controlling the temperature
- Synergism with bactericocin and bactericidal compounds

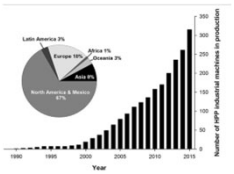


High Pressure Processing, Public Health Microbiology Laboratory

Information about the units: https://ir.pressurebiosciences.com/press-releases/detail/284/pressure-biosciences-announces-commercial-release-of-the



Allison et al., 2018



High Pressure Processing, Public Health Microbiology Laboratory

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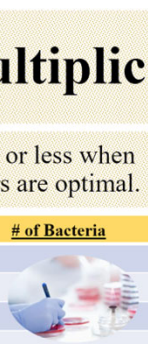
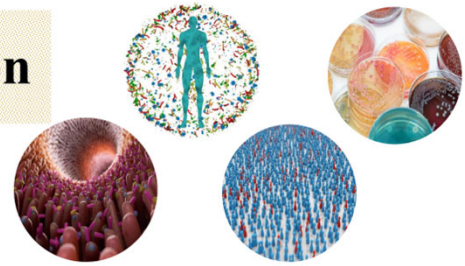
Bacterial Multiplication

Binary Fission: 20 minutes or less when intrinsic and extrinsic factors are optimal.

Time	# of Bacteria
0 minutes	1
20 minutes	2
40 minutes	4
1 hour	8
2 hours	64
4 hours	4,096
6 hours	262,144
8 hours	16,777,216
12 hours	68,719,476,736





Bacteria	Estimated Infective Dose*
Salmonella serovars	<10 cells
Shiga toxin-producing *E. coli*	10 to 100 cells
Cronobacter sakazakii	10 to 100 cells
Listeria monocytogenes	<1000 cells
Campylobacter spp.	5000 to 10,000 cells
Staphylococcus aureus	>100,000 cells
Vibrio cholerae	1,000,000 cells

Information and photos are modified and adopted from BBB of Food and Drug Administration, BAM Resources of Centers for Disease Control and Prevention. Photo Courtesy: Adobe Stock (standard license of photos purchased by the Public Health Microbiology laboratory).

** Calculated for oral ingestion based on epidemiological data from outbreaks and human feeding trials of volunteers. Data obtained from BBB of Food and Drug Administration (2nd edition).*

Public Health Microbiology Laboratory: Education, Research, Outreach, and Technical Assistance: https://publichealthmicrobiology.education/

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Emerging pathogens

Vertical and Horizontal Gene Transfer and Emerging Pathogens

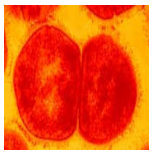
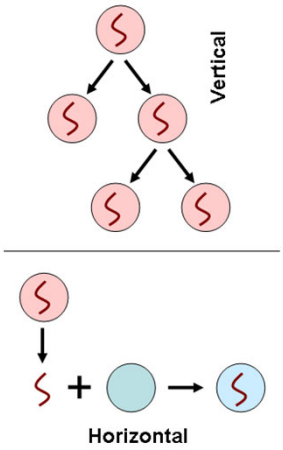
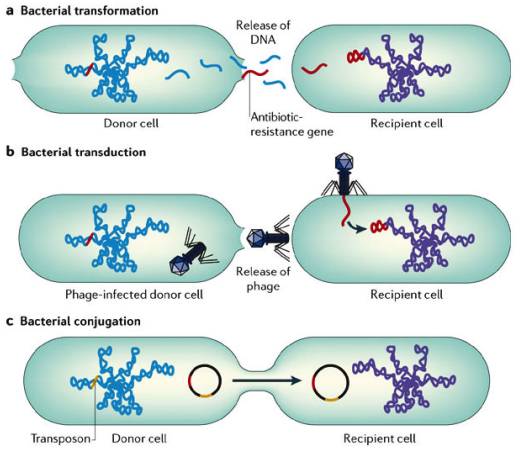


Photo Courtesy:
http://www.daviddarling.info/encyclopedia/B/binary_fission.html





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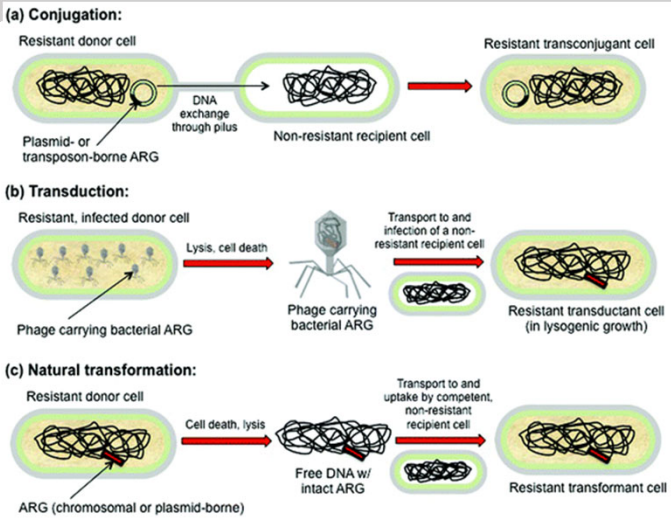
Horizontal Gene Transfer



Donn, 2012

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Planktonic cells and Biofilm Communities

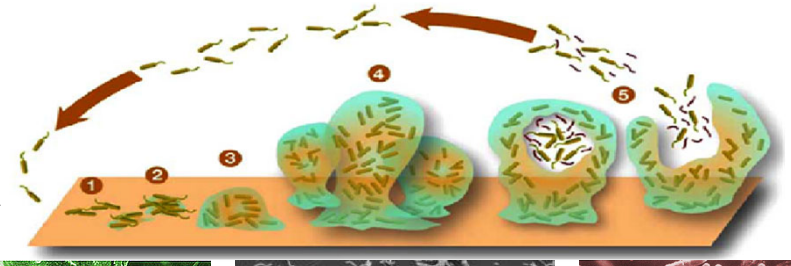

Photo Courtesy: http://prometheus.matse.illinois.edu/glossary/biofilms/

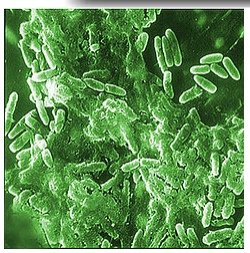

Photo Courtesy: http://micro-writers.egybio.net/blog/?tag=antibiotic-resistance

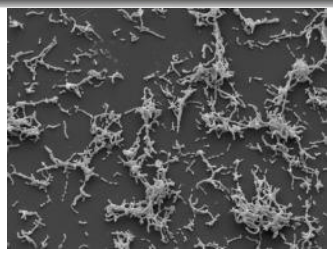

Photo Courtesy: http://www.ifenergy.com/50226711/boosting_microbial_fuel_cells_with_biofilm.php

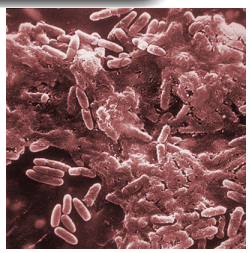

Photo Courtesy: http://www.microbiologybytes.com/blog/category/biofilms/

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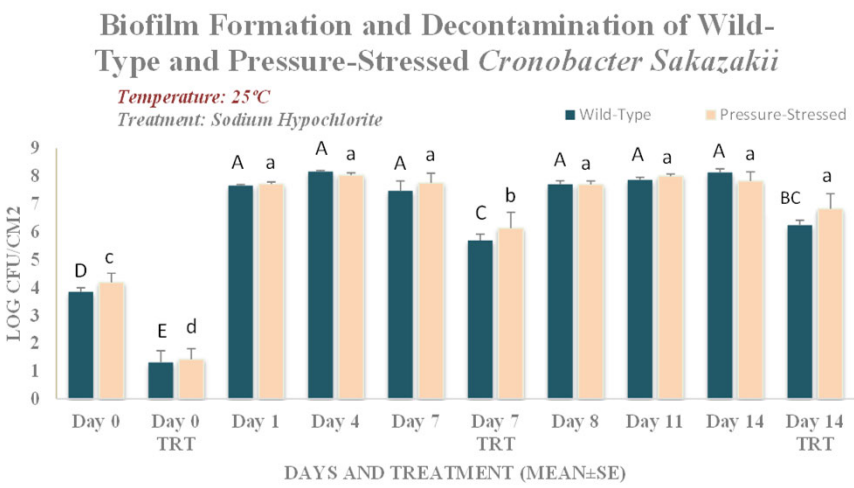
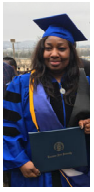
Cronobacter sakazakii
Two outbreaks in Tennessee (1998, Memphis; 2001 Knoxville)





Allison et al., 2020

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Quorum Sensing and Biofilm formation

Shiga toxin producing *E. coli*, not antibiotic treatment due to Quorum Sensing Concerns

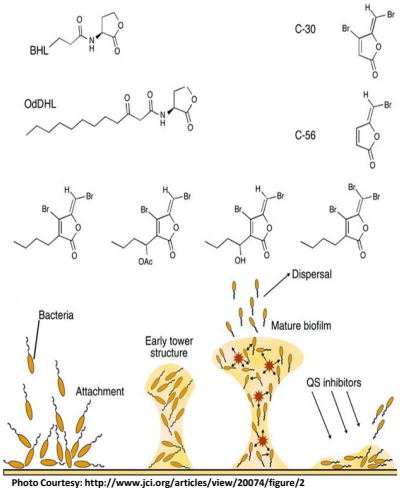

Photo Courtesy: http://www.jci.org/articles/view/20074/figure/2

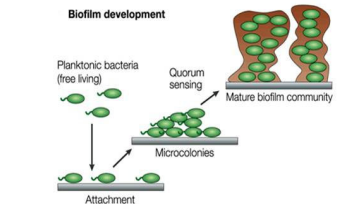

Photo Courtesy: http://labrat.fieldofscience.com/2010/07/quorum-sensing-and-biofilms.html

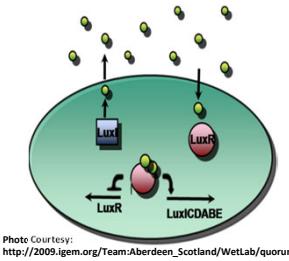

Photo Courtesy: http://2009.igem.org/Team:Aberdeen_Scotland/WetLab/quorumsensing

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Infectious Diseases is a Moving Target…

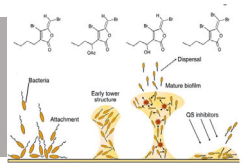


Photo Courtesy: http://www.jci.org/articles/view/20074/figure/2

Yersinia pestis 14th Century Outbreak in Europe, c. 30 to 50% of the population

Antoni van Leeuwenhoek: Discovery of bacteria in 1676 (c. 350 years)

Viruses discovered in 1890s

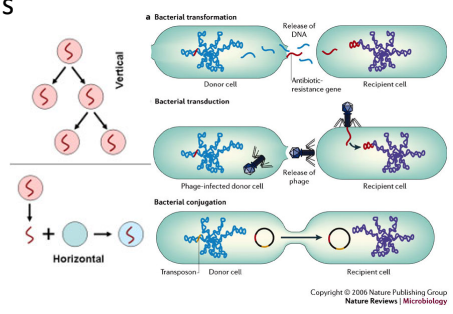
- It is estimated only 1% of microbial community has been identified.
- Currently etiological agent of 80.3% of foodborne illnesses, 56.2% of hospitalization, and 55.5% of deaths remain unknown.

3.5 billions vs. 300,000 years

"Emerging" Pathogens:

- Vertical and horizontal gene transfer spores and biofilm formation
- Quorum sensing and cell to cell communication

"*It is the microbes who will have the last word.*"
-Louis Pasteur



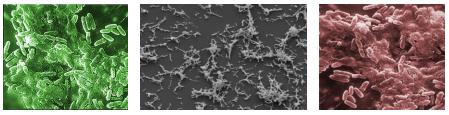
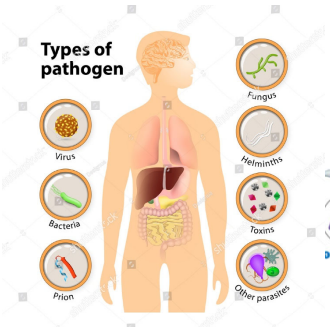


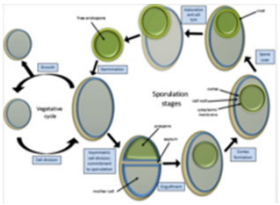
Photo Courtesy: http://www.microbiologybytes.com/blog/category/biofilms/
http://www.ifenergy.com/50226711/boosting_microbial_fuel_cells_with_biofilm.php
http://micro-writers.egybio.net/blog/?tag=antibiotic-resistance

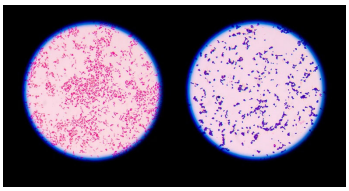
12

Anthrax

- Causative agent: *Bacillus anthracis*
- A **Gram-positive** and **spore-forming** bacteria
- Can be found as a spore in the **soil worldwide**
- Spores **viable** for **decades in soil**
- Common in parts of Africa, Asia, and Middle East
- In Human:
  - Skin
  - Intestine
  - Inhalation
- Animal disease
  - Septicemia and rapid death







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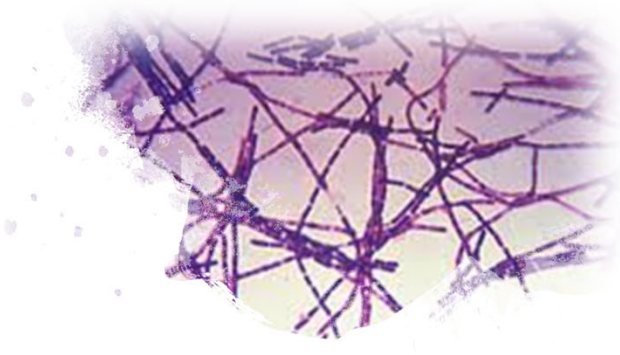
Anthrax

- Spores highly infective
- Remain effective during aerosolization
- Low lethal dose
- High mortality
- Person-to-person transmission rare
- **Symptoms** begin between **one day** and **two months** after the infection



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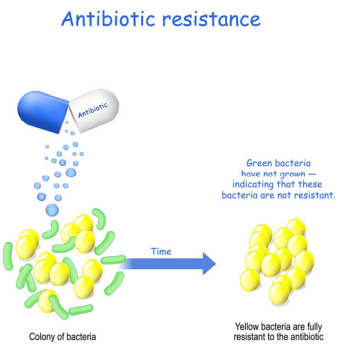
Anthrax- Control and Treatment

- Vaccine for livestock annually to prevent
- Personal Protective Equipment
  - When handling sick animals
- Disinfection:
  - **Sporicidal agents:** 5% formaldehyde, 2% glutaraldehyde, 10% sodium hydroxide
  - **Sterilization:** chlorine dioxide, formaldehyde gas, heating to **121 ºC** for at least 30 minutes
- **Antibiotics:** effective for humans when **prescribed early**



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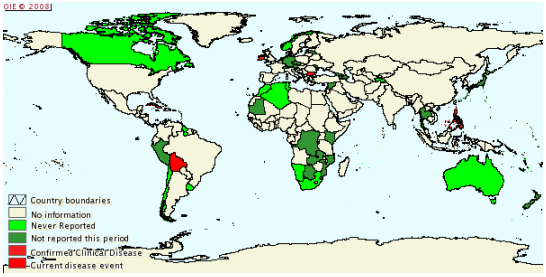
Pseudorabies

- **Contagious** viral diseases from **herpes family**
- Primary concern in domesticated **pigs and feral swine (around 75 million hogs in the United States in 2021)**
- Primarily spread through direct **animal-to-animal** (nose-to-nose)
- **Other mammals**
  - Reproductive
  - Nervous system
- **Humans are not affected**
- Could be a **ubiquitous virus** in some area
- **Eradicated in many countries**
  - Still occurs in parts of world
- Current **USDA Surveillance** to detect any potential case

-Different than rabies that is an important zoonotic diseases.
-Rabies death in the U.S. now < 5 per year
-About 59,000 annually worldwide (>98% from stray dogs)
Source: CDC, 2021



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Pseudorabies

- Transmission:
  - Direct contact,
  - Reproductive,
  - Aerosol,
  - Ingestion
- Incubation period: 2-6 days (for COVID-19 currently believed to be 2 to 14 days, CDC, 2021)
- Common symptoms:
  - Neurological
  - Respiratory issues
  - Itching intensively
  - Stillbirths and abortion
- Morbidity and mortality up to 100%
- Neonates are particularly susceptible to the virus

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Foot-and-Mouth Disease

- Highly communicable **viral disease** (*Aphthovirus* of the family Picornaviridae).
- Livestock hosts:
  cattle, pigs, sheep, goats (experimental infections in alpacas and llamas).

Symptoms:

- fever
- vesicles in the **mouth and on the muzzle, teats, and feet**.
- In susceptible population, **morbidity reaches 100%**

Transmission:

Spreads through **direct contact** or **aerosolized virus** via:

- respiratory secretions
- milk
- semen
- ingestion of feed from infected animals (meat, offal, milk).

Source: Merck Veterinary Manual

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Foot-and-Mouth In the United States

- US had total of **9 FMD outbreaks** in addition to many **sporadic cases**
- **Historic outbreak and some of the earliest regulation:**
- 1870, 1880 and 1884: **imported** animals, lead to **federal inspection and quarantine programs** that had been successful to preventing the disease to occur from imported animals since 1884.
- 1902, 1908, 1914, 1924 (twice) and 1929 from pathogen existing on **mainland**
- **The six later were controlled by**: stopping movement and **stamping out**
- Currently only **sporadic cases** in north America that is controlled aggressively with stamping out

Source: Center for Food Security and Public Health, Iowa, Zoonotic Diseases
Source: Merck Veterinary Manual

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Foot-and-Mouth- Global Prevalence

World Organization for Animal Health (OIE):

- 178 member countries
- 96 countries are endemic (never been free of FMD
- 66 countries free of FMD
- 11 countries have free zones (with or without vaccination)
- 5 countries were free, recently suffered from re-emergence of FMD

Source: Center for Food Security and Public Health, Iowa, Zoonotic Diseases
Source: Merck Veterinary Manual

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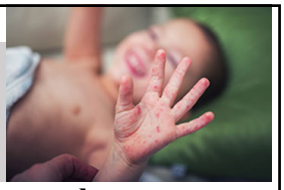
Evolving Practices in Handling FMD Outbreak

- Stop movement
- Stamping Out
- **Slaughter of all clinically affected** and in-contact susceptible animals (within 24 hours or as soon as possible)
- **Trace back/Trace forward epidemiology**: 28 days prior to outbreak
- **Rapid Diagnostics**
- **Vaccination** (Vaccinate to kill/slaughter; Vaccinate to live)

Source: Center for Food Security and Public Health, Iowa, Zoonotic Diseases
Source: Merck Veterinary Manual

21

Burden of the Disease

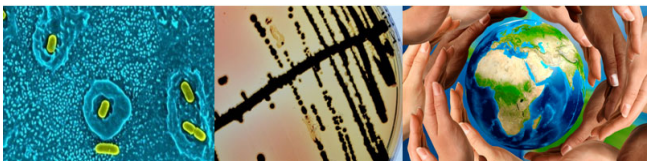


- Animals at risk in the United States
  - 100 million cattle
  - 60 million swine
  - 7 million sheep
  - 40 million wildlife
  - Not horses
- Humans rarely infected
- Huge economic impact

- **Hand, foot, and mouth disease** is a common viral illness that usually **affects infants and children younger than 5 years** old. However, it can sometimes occur in older children and adults
- There is **no specific treatment** for hand, foot, and mouth disease. However, you can do some things to relieve symptoms: OTC **pain relieve** and mouthwash to reduce symptoms

Animal Disease Emergencies, 2008 - IHSEMD, IDALS, CFSPH; https://www.cdc.gov/hand-foot-mouth/index.html; Photo courtesy: CDC

22



Foodborne Diseases of Public Health Importance

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Epidemiology of Foodborne Diseases in the United States

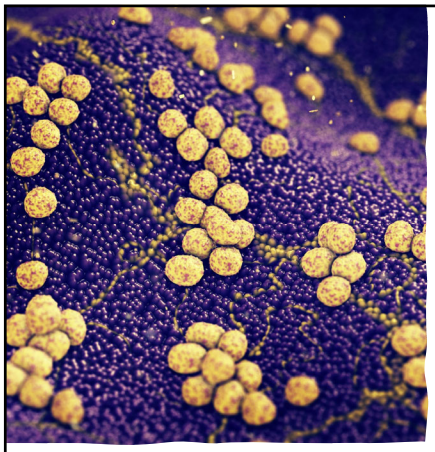
Based on data from 1990s: *(Mead et al., 1999)*

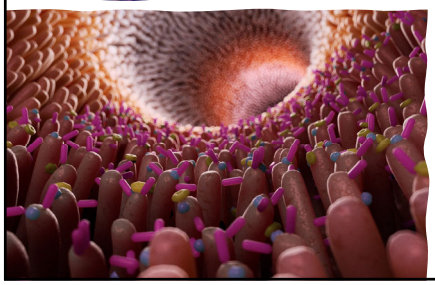
76 million illnesses, 323,000 hospitalizations, 5,200 deaths in the United States.

More recent estimates show: *(Scallan et al., 2011)*

- **47.8 million illnesses**, 127,839 hospitalizations, and more than **3,037** deaths in the United States. **(c. 1.7M cases 300K deaths/year of sepsis)**
- 9.4 million illnesses, 55,961 hospitalizations, and 1,351 deaths are cause by 31 known foodborne agents.
- In addition to consumer insecurity, foodborne diseases cause around $**77.7 billion** for losses in productivity and economical losses. **(2021 GPD of Jamaica 14.66 Billion)**
- Approximately 30% of population are especially "at risk" for foodborne diseases (The **YOPI's**: The young, the old, Pregnant, and Immunocompromised)

24





Signs and Symptoms of Foodborne Diseases

- Mild illness (no medical care sought)
- **Guillain–Barré syndrome** (*Campylobacter* and *Salmonella*)
- **Post-infectious irritable bowel syndrome** (*Campylobacter* and *Salmonella*)
- **Reactive arthritis** (*Campylobacter* and *Salmonella*)
- **Haemolytic uraemic syndrome** (*E. coli* O157)
- **End-stage renal disease** (*E. coli* O157)
- Death

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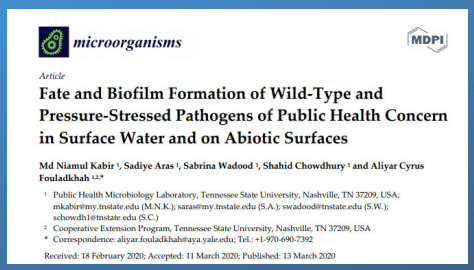
Water Safety Study



microorganisms

MDPI

Article

Fate and Biofilm Formation of Wild-Type and Pressure-Stressed Pathogens of Public Health Concern in Surface Water and on Abiotic Surfaces

Md Niamul Kabir [1], Sadiye Aras [1], Sabrina Wadood [1], Shahid Chowdhury [1] and Aliyar Cyrus Fouladkhah [1,2,*]

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[2] Cooperative Extension Program, Tennessee State University, Nashville, TN 37209, USA
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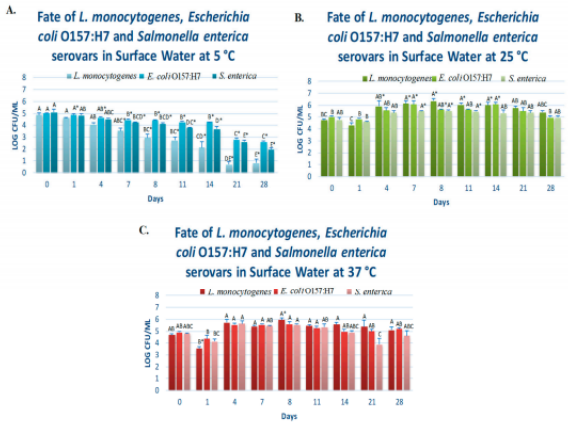
Public Health Burden of Waterborne Disease

17 waterborne pathogens cause estimated: (Collier et al., 2021)

601,000 **illness**; 118,000 **hospitalization**; 6,630 **deaths**, and cost the economy up to $ 8.77 **billions.**



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Significant foodborne pathogens…
based on Mead et al., 1999 and Scallan et al., 2011 studies

- **Leading etiological agents for illnesses**: *Norovirus* (58%), Nontyphoidal *Salmonella* serovars (11%), *Clostridium perfringens* (10%), and *Campylobacter* spp (9%).
- **Leading etiological agents for hospitalization:** Nontyphoidal *Salmonella* serovars (35%), *Norovirus (26%), Campylobacter* spp (15%), and *Toxoplasma gondii* (8%).
- **Leading etiological agents for death:** Nontyphoidal *Salmonella* serovars (28%), *T. gondii* (24*%), Listeria monocytogenes* (19%), and *Norovirus (11%).*

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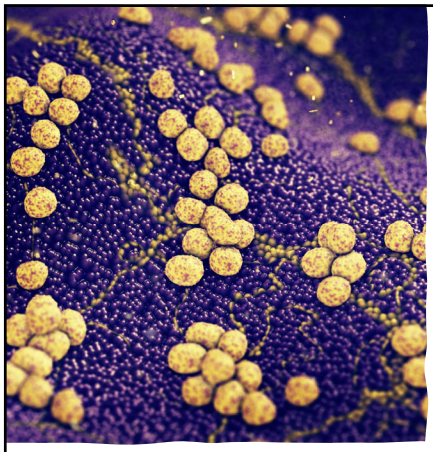

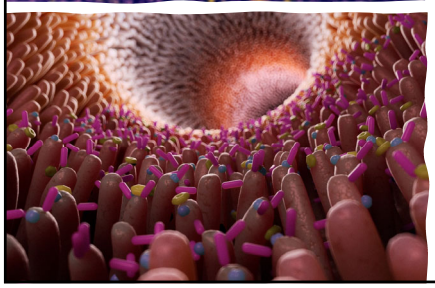

Signs and Symptoms of Foodborne Diseases

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- **Haemolytic uraemic syndrome** (*E. coli* O157)
- **End-stage renal disease** (*E. coli* O157)
- Death

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Significant foodborne pathogens…
based on Scallan et al., 2015 study

- **Disability adjusted life year** (DALY). *DALY: Loss of life and health due to illness*
- Non-typhoidal *Salmonella* (329000)
- Toxoplasma (32700)
- *Campylobacter* (22500)
- Norovirus (9900)
- *Listeria monocytogenes* (8800)
- *Clostridium perfringens* (4000)
- *Escherichia coli* O157 (1200)

One DALY can be thought of as one **lost year of "healthy" life**.

DALY= YLL+YLD

YLL: Years of Life Lost (YLL) due to **premature mortality** in the population
YLD: Years Lost due to Disability (YLD) for **people living with the health condition**

Source: WHO, 2019

62% bacterial agents; 29% parasitic agents; 9% viral agents

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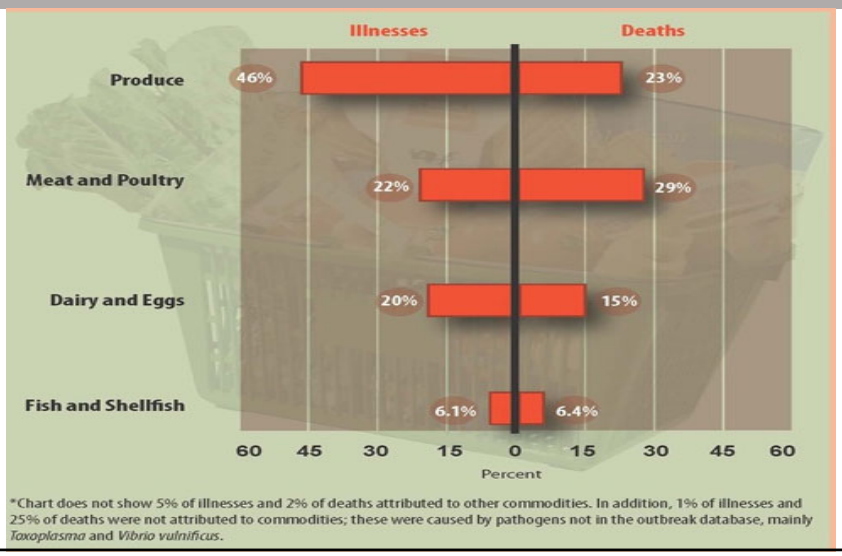
CDC Estimates of Food Safety Burden

http://www.cdc.gov/foodborneburden/attribution-image.html#foodborne-illnesses



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Are these outbreaks associated with corporates and lager manufactures?

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Prevalence of Pathogens in Medium-sized Poultry Operations

- 200–300 ft houses, 3000 to 5000 birds, conventional operation

(Alali et al., 2010)

	Salmonella serovars
Fecal samples (n=420)	38.8%
Feed (n=140)	27.5%

- Total of 135 sample from commercial free-range chicken producers

(Bailey et al., 2005)

	Salmonella serovars
Chicken Carcasses in Operation 1	64%
Chicken Carcasses in Operation 2	31%

Alali et al., 2010, J Foodborne Pathogens and Diseases; Bailey et al., 2005, J Food Protection

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Prevalence of Pathogens in Small Poultry Farms

- Study of 60 Small poultry slaughterhouses (fewer than 200 birds slaughtered per day)

Sampling sites	*Salmonella* serovars (Albany, Hadar, Indiana, and Enteritidis sub-species)
Carcasses after slaughter	42%
Utensils	23.1%
Storage freezers and refrigerators	71.4%

- The Study concluded "*The widespread occurrence of Salmonella in small slaughterhouses reinforces the need for implementation of effective control measures…*"

Terumi et al., 2000, Journal of Food Protection

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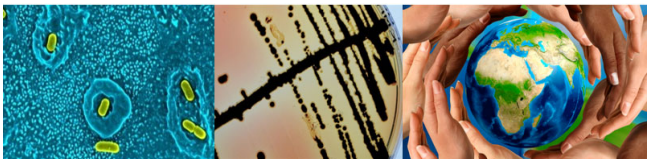
Foodborne Pathogens of Public Health Concerns



- *Salmonella* serovars
- *Staphylococcus aureus*
- *Campylobacter* spp.
- *Bacillus cereus*
- Shiga Toxin-Producing *Escherichia coli* (STEC)
- *Vibrio* spp.
- *Yersinia enterocolitica*
- *Streptococcus* spp.
- *Shigella* spp.
- *Listeria monocytogenes*

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Salmonella serovars

- **Annual illness (death): 1,027,561 (378) in humans**
- **Infection** causes nausea, vomiting, diarrhea, fever, headache
- **Primary sources**: Intestinal tract of people and animals
- **Transmitted by** meat, poultry, eggs, raw milk, unpasteurized juice, many other foods (nuts, spices, produce, chocolate, flour)
- **Contributing factors**: cross-contamination, undercooked food, poor agricultural practices

Growth parameters	Minimum	Optimum	Maximum
Temperature	41°F (5.2°C)	95-109°F (35-43°C)	115°F (46.2°C)
pH	3.7	7-7.5	9.5
a_w	0.94	0.99	>0.99
Other	Non-spore former		
Atmosphere	Facultative - grows with or without oxygen		

Sources: ICMSF 1995 and Bad Bug Book 2nd edition, Scallan et al., 2011, and FSPCA

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Salmonella serovars

- **Carriers**: Reptiles (turtles, lizards, and snakes); Amphibians (frogs and toads); Poultry (chicks, chickens, ducklings, ducks, geese, and turkeys); Other birds (parakeets, parrots, and wild birds); Rodents (mice, rats, hamsters, and guinea pigs); Other small mammals (hedgehogs); Farm animals (goats, calves, cows, sheep, and pigs); Dogs; Cats; Horses.
- **Dogs and cats** that become ill from *Salmonella* infection generally will have diarrhea that may contain blood or mucus
- Some **cats** do not have diarrhea, but will have a **decreased appetite, fever, and excess salivation**.

Prevention:

- **Minimizing direct contact**, **washing hands**, and **cleaning up** after the pets could minimize the risk of transmission from infected animals to human.

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Climate Change and Pubic Health Microbiology

Non-typhoidal *Salmonella enterica* serovars

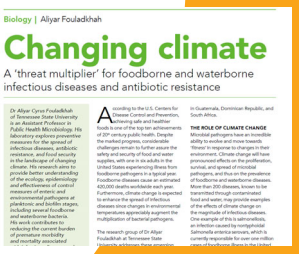
- **Global death:** 50,000 global death in 2010 (WHO, 2020)
- **Public Health Burden in the U.S.:** >1 million annual cases in 2011 (CDC, 2011)

Climate Change:

- **1 °C increase : 5 to 10% increases in Salmonellosis (WHO, 2010)**
- 2500 to 5000 additional global death
- 50,000 to 100,000 U.S. morbidity

At our current rate:

- >1.5 °C by 2040
- >4.8 °C by 2100



Biology | Aliyar Fouladkhah

Changing climate

A 'threat multiplier' for foodborne and waterborne infectious diseases and antibiotic resistance

microorganisms

MDPI

Editorial

Safety of Food and Water Supplies in the Landscape of Changing Climate

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3 Department of Civil and Environmental Engineering, Vanderbilt University, Nashville, TN 37235, USA; janey.camp@vanderbilt.edu
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check for updates

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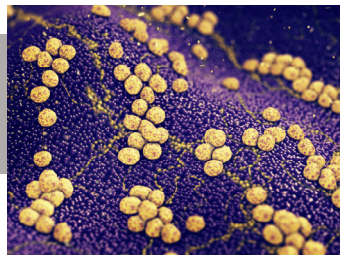
Staphylococcus aureus



- **Annual illness (death): 241,148 (6)**
- Both causes infection and toxico-infection
- Produces heat stable toxins after extensive growth
- **Primary sources**: Boils, nasal passages and skin *(some studies >15%)*
- **Transmitted** by recontaminated cooked foods, and foods with high salt (halophilic bacteria) or high sugar
- **Contributing factors**: Recontamination and temperature abuse

Growth parameters	Minimum		Optimum		Maximum	
	Growth	Toxin	Growth	Toxin	Growth	Toxin
Temperature	45°F (7°C)	50°F (10°C)	99°F (37°C)	104-113°F (40-45°C)	122°F (50°C)	118°F (48°C)
pH	4	4	6-7	7-8	10	9.8
a_w	0.83	0.85	0.98		>0.99	
Other	Poor competitor, non-spore former					
Atmosphere	Facultative – grows with or without oxygen, but slower without					

Sources: ICMSF 1995 and Bad Bug Book 2nd edition, Scallan et al. 2011, and FSPCA

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Staphylococcus aureus

- Methicillin-resistant *Staphylococcus aureus* (MRSA) a **major concern for animal and human health**.
- Humans **working closely in animal** feeding operations are in elevated risk of exposure to this pathogen particularly the **antibiotic resistant phenotypes**
- A contagious bacterium responsible for vast majority of environmental **Mastitis in dairy** operations.
- Cause of "bumblefoot" in chickens
- A major pathogen of farm rabbits

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Campylobacter spp.

- **Annual illness (death): 845,024(76)**
- Infection causes diarrhea, and potential nerve damage
- **Primary sources**: Intestinal tract of animals
- **Transmitted** by raw poultry, raw milk products, contaminated water, poultry
- **Contributing factor**: cross contamination and undercooking

Growth parameters	Minimum	Optimum	Maximum
Temperature	86°F (30°C)	108-109°F (42-43°C)	113°F (45°C)
pH	4.9	6.5-7.5	9.5
a_w	>0.987	0.997	-
Other	Non-spore former		
Atmosphere	3-5% oxygen optimum		

Sources: ICMSF 1995 and Bad Bug Book 2nd edition and FSPCA

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Campylobacter spp.

- Bacterium exist in GI track of **many healthy farm and companion** animals.
- **Dogs six week and younger** are prone to Campylobacteriosis with symptoms:
- -Fever
- -Vomiting
- -Loss of appetite
- -Enlarged lymph nodes
- **Staying in kennels that increases the exposure to fecal matter** and contact with contaminated food and water are main sources of disease in dogs.

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Shiga Toxin-Producing *Escherichia coli* (STEC)

- **Annual illness (death): 176,152 (20)**
- **Infection causes** bloody diarrhea, and sometimes kidney failure and death *(kids under the age of 5)*
- **Primary sources**: Intestinal tract of ruminant animals (e.g., cows, sheep)
- **Transmitted** by raw and undercooked beef, poultry, leafy greens, and unpasteurized milk and juices
- **Contributing factors**: poor GAP, inadequate heating, and person-to-person

Growth parameters	Minimum	Optimum	Maximum
Temperature	44°F (6.5°C)	95-104°F (35-40°C)	121°F (49.4°C)
pH	4	6-7	10
a_w	0.95	0.995	-
Other	Non-sporeforming		
Atmosphere	Facultative - grows with or without oxygen		

Sources: ICMSF 1995 and Bad Bug Book 2nd edition,Scallan et al. 2011, and FSPCA

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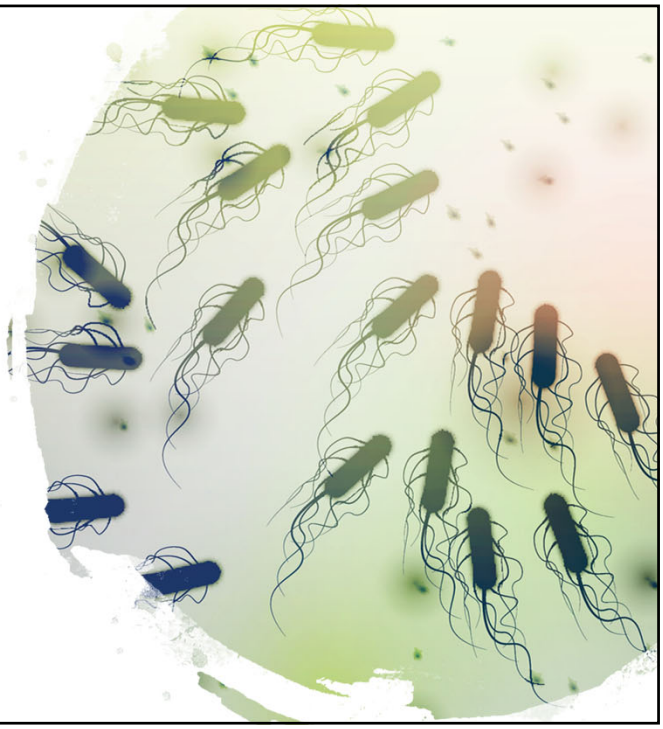
Shiga Toxin-Producing *Escherichia coli* (STEC)

- **Animals that can spread *E. coli* O157 to humans include**:
  - -cows, especially calves
  - -goats
  - -sheep
  - -deer
- *E. coli* infection very common in **cats and puppies younger than one week**.
- **Colostrum**, plays a pivotal role in protecting a newborn the animal's undeveloped immune system against *E. coli* infection.
- As high as **80% of agricultural animals could carry various serogroups of shiga-toxigenic *E. coli*** without having symptoms



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Quorum Sensing and Biofilm formation

Shiga toxin producing *E. coli*, not antibiotic treatment due to Quorum Sensing Concerns

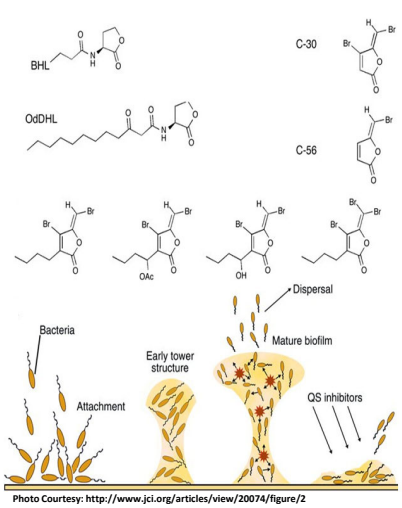

Photo Courtesy: http://www.jci.org/articles/view/20074/figure/2

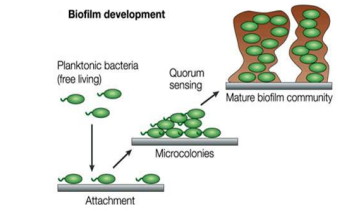

Photo Courtesy: http://labrat.fieldofscience.com/2010/07/quorum-sensing-and-biofilms.html

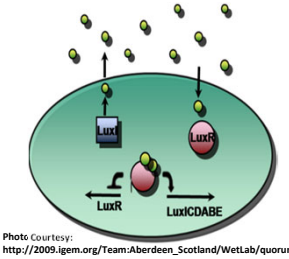

Photo Courtesy: http://2009.igem.org/Team:Aberdeen_Scotland/WetLab/quorumsensing

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Vibrio spp.

- Causing about **80,000 illness and 100 death** annually in the United States.
- **Infection symptoms** vary depending on strain, ranging from diarrhea to high fever
- Vibrio is a **halophilic bacterium** and is a major concern in aquaculture industry
- **Primary sources**: Salt water environments and seafood
- Requires salt to reproduce (halophile)

Growth parameters	Minimum	Optimum	Maximum
Temperature	41°F (5°C)	99°F (37°C)	114°F (45.3°C)
pH	4.8	7.8-8.6	11
a_w	0.94	0.98	0.996 (10% NaCl)
Other	Non-sporeformer, requires salt		
Atmosphere	Facultative - grows with or without oxygen		

Sources: Seafood Hazards Guide 2011, ICMSF 1995 and Bad Bug Book 2nd edition

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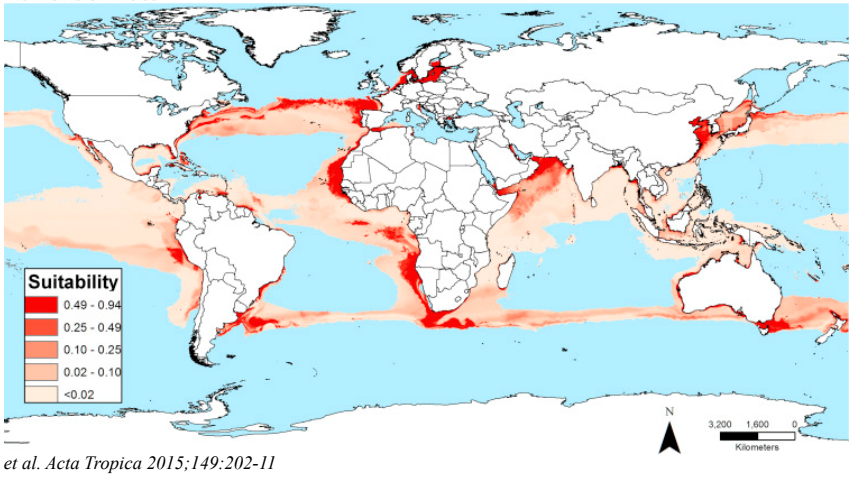
Vibrio cholerae proliferation in sea water: Current Climate

***Vibrio Cholerae*: currently 760,000 global illness/24,000 death per year**

Current climate



Escobar LE et al. Acta Tropica 2015;149:202-11

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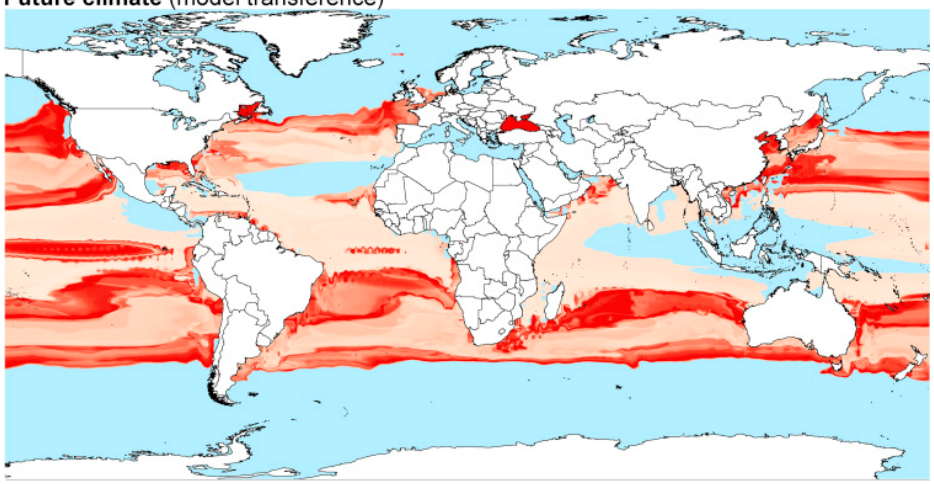
Vibrio cholerae proliferation in sea water: *Business-as-Usual Projection in 2100*

Future climate (model transference)



Escobar LE et al. Acta Tropica 2015;149:202-11

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Yersinia enterocolitica

- **Infection causes** abdominal pain, fever and diarrhea. May mimic appendicitis.
- **Primary sources**: Raw pork, raw milk
- **Contributing factors**: Cross contamination between raw pork products and RTE foods
- Causes only **mild clinical signs in cats and dogs of short duration**, usually with no mortality

Yersinia pestis 14th Century Outbreak in Europe, c. 30 to 50% of the population

Antoni van Leeuwenhoek: Discovery of bacteria in 1676 (c. 350 years)

Viruses discovered in 1890s

Growth parameters	Minimum	Optimum	Maximum
Temperature	30°F (-1.3°C)	77-99°F (25-37°C)	108°F (42°C)
pH	4.2	7.2	10
a_w	0.945	-	-
Other	Non-sporeformer		
Atmosphere	Facultative - grows with or without oxygen		

Sources: Seafood Hazards Guide, ICMSF 1995, and Bad Bug Book

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Listeria monocytogenes

- **Infection causes** severe illness in susceptible people – mortality 15-30%
- **Primary sources**: Occurs widely in agriculture (soil, plants and water)
- **Transmitted by**: Refrigerated RTE foods that support growth *(pathogen of concern during pregnancy)*
- **Contributing factors**: Environmental (ubiquitous) pathogen spread by environmental contamination, equipment, people, incoming raw ingredients (in absence of Gram-negative bacteria?)
- **Common in domesticated ruminates particularly** sheep, poultry, and birds.
- **Could cause sporadic and farm outbreaks in ruminants**
- **Could cause: Encephalitis, late abortion, and GI problems in ruminants**.

Growth parameters	Minimum	Optimum	Maximum
Temperature	31°F (-0.4°C)	99°F (37°C)	113°F (45°C)
pH	4.4	7.0	9.4
a_w	0.92	-	-
Other	Non-sporeformer		
Atmosphere	Facultative - grows with or without oxygen		

Sources: ICMSF 1995 and Bad Bug Book 2nd edition

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Additional Resources and References:

Centers for Disease Control and Prevention:

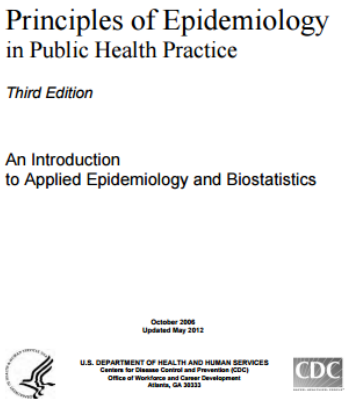
https://www.cdc.gov/ophss/csels/dsepd/ss1978/ss1978.pdf

Center for Food Security and Public Health, Iowa, Zoonotic Diseases:

http://www.cfsph.iastate.edu/Zoonoses/

Food and Agriculture Organization of the United Nation:

http://www.fao.org/emergencies/emergency-types/transboundary-animal-diseases/en/



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Bad Bug Book

Bad Bug Book (Second Edition)

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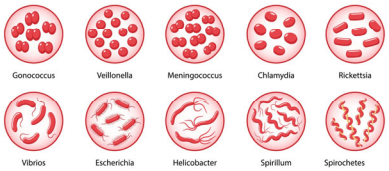
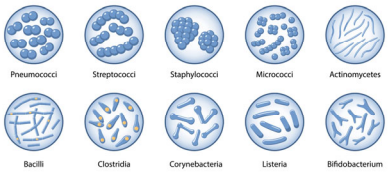
Foodborne Pathogenic Microorganisms and Natural Toxins Handbook

Gram negative bacteria



Gram positive bacteria



Bad Bug Book

Handbook of Foodborne Pathogenic Microorganisms and Natural Toxins

Introduction

Food safety is a complex issue that has an impact on all segments of society, from the general public to government, industry, and academia. The second edition of the Bad Bug Book, published by the Center for Food Safety and Applied Nutrition, of the Food and Drug Administration (FDA), U.S. Department of Health and Human Services, provides current information about the major known agents that cause foodborne illness. The information provided in this handbook is abbreviated and general in nature, and is intended for practical use. It is not intended to be a comprehensive scientific or clinical reference.

Under the laws administered by FDA, a food is adulterated if it contains (1) a poisonous or otherwise harmful substance that is not an inherent natural constituent of the food itself, in an amount that poses *a reasonable possibility* of injury to health, or (2) a substance that is an inherent natural constituent of the food itself, is not the result of environmental, agricultural, industrial, or other contamination; and is present in an amount that *ordinarily* renders the food

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Thank you

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FARMER TO FARMER
The USAID John Ogonowski and Doug Bereuter Farmer-to-Farmer Program

Volunteer Certification Form (VCF)

Volunteer and Field Staff complete and sign the VCF prior to departure → Field Staff scan and email the VCF to HQ PC → PC checks the information, signs and scans the VCF → PC enters data into the VCF form in NOVAS, uploads the original to the M&E subsite

- Submit one VCF per assignment
- This VCF is subject to examination by USAID and ACDI/VOCA's external auditors. Please be as specific as possible and provide back-up documentation (training attendance sheets, proof of third-party contributions, etc.) to the Farmer-to-Farmer (F2F) Field Staff
- Volunteer submits the Final Report to the Country Director and F2FVolunteer@acdivoca.org within two weeks after the assignment, if not submitted in-country (see template in the Welcome Packet)
- Volunteer submits the Volunteer Expense Report (VER to F2FVolunteer@acdivoca.org with scanned receipts and boarding passes within 30 days of assignment (see template in the Welcome Packet)
- Information related to outreach activities in the U.S. (media appearances, presentations, etc.) will be collected through a bi-annual survey distributed separately via Survey Monkey.

Assignment Data – To Be Completed by the Volunteer

Assignment Number (refer to the Scope of Work):	GEO-2014-079
Volunteer Name:	Dr. Aliyar Cyrus Fouladkhah
Country:	Georgia
Assignment Title:	Training in Public Health Microbiology
Host Organization(s):	AFDA
Assignment Dates:	4/18/2023-5/2/2023
PERSUAP Number, if applicable (refer to the Scope of Work):	N/A

Number of Persons Trained (include training attendance sheets):

Male	**2**
Female	**3**
Non-Binary	**0**
Out of these: - Youth (Ages 15-29)	**0**

Volunteer Certification Timesheet

Type	Dates (Range)	# of Days
Pre-Assignment Days (1 Day = 8 Hours) *If more than 3 pre-assignment days, please provide a description of activities	4/15-4/18/2023	3
International Travel to Assignment	4/18/2023-5/19/2023	2
Assignment Days	4/20/2023-5/2/2023	13
International Travel Home	5/3-5/3/2023	1
Personal Days	**0**	

USAID FROM THE AMERICAN PEOPLE

ACDI VOCA Expanding Opportunities Worldwide

Total # of Days	19

Number of Recommendations, per Host (no more than 6 per host)

1. Implementation of microbial analyses with the supplies provided to the entrepreneur from Public Health Microbiology Foundation for analysis of food and water samples.
2. Implementation of microbiological testing thought to the entrepreneurs using the provided supplies for isolation of samples from the veterinary clinic and implementing the antimicrobial stewardship program discussed using both Gram-staining and antibiotic susceptibility tests.
3. Adherence to biosafety guidelines thought to the members of the laboratory for ensuring the safety of the lab personal, stakeholders, and the community.

Did you conduct any radio or TV broadcasts or publications while in the host country?
If yes, please explain: N/A

Additional Contributions – To Be Completed with Field Staff

I. Host Contributions

If the host contributed any resources to assist with the volunteer assignment, please list them here:

Description	Unit Value USD	# of Units	Total
Meals – Breakfasts	$ 0		
Meals – Lunches	$15	7	$ 105
Meals – Dinners	$	0	$
Lodging – Nights	$ 0	0	$
Interpreters – Days	$		$
Transportation – Days	$30	8	$240
		Total of Contributions, Host 1	$ 345
		Total of Contributions, Host 2	$
		Total of Contributions, Host 3	$

II. Volunteer Contributions – *Please include receipts in the Volunteer Expense Report*

*** Items were financially support by the Public Health Microbiology Foundation in Nashville, TN (Founding Director, Dr. Aliyar Cyrus Fouladkhah).**

Description of Item	Unit Value USD	# of Units	Total
Microbiology Laboratory supplies, media, and consumables	**c. $2,500 (conservatively estimated)**	**28 Items**	**$2,500**
		Subtotal:	**$2,500**
		(Minus the amount to be reimbursed to the Volunteer)	
		Total Contributions:	**$2,500**

III. Partner or Third-Party Contributions in the U.S.

If a partner or a third party in the United States (your hometown community, business group, church, etc.) provided a contribution that you brought to the host(s), please list it here.

Contributor	Description of Item	Unit Value in USD	# of Units	Total
			Total of Contributions in USD	

Assignment Summary – To Be Completed by Field Staff

Dr. Aliyar Cyrus Fouladkhah brought with himself laboratory supplies, media, and consumables for establishing microbiological procedures in the host institution in Zugdidi. Thanks to these suppliers, during the assignment, laboratory procedures were established for conducting microbiological analysis of veterinary clinic samples as well as various foods and water samples. One individual with a doctorate and the other, a doctorate candidate, were extensively trained for the conduct of these microbial analyses. Additionally, a comprehensive antibiotic stewardship program was established for rapid selection (within one hour) of the most potentially efficacious antibiotic (on Gram-staining bases) and antibiotic susceptibility test withing 24 to 48 hours. Several microbial samples including water and meat products obtained from the local market were analyzed by trainees of this assignment. The entrepreneur received extensive training about infectious disease in their region, examination of various bacterial and mammalian tissues (selection of 100 microscope slides were donated from the Public Health Microbiology Foundation), microbiological analyses, and antibiotic stewardship. Extensive training was also conducted in biosafety to ensure the trainees and the host institution could ensure the safety of the lab members, the stakeholders, and the community. This assignment was approximate 80% hands-on training and consultation in laboratory and 20% lecture-based traditional training.

In summary these trainings were provided:

- Food Microbiology Analysis
- Water Safety Analysis
- Mammalian Tissue and Microbiology Examination Under Microscope
- Antibiotic Stewardship (Gram-staining and Antibiotics susceptibility test)
- Infectious Diseases of Prevalence in Georgia and Zoonotic
- Biosafety Training for Microbiology Laboratory

PERSUAP Table (if applicable) – To Be Completed by Field Staff

If you interacted with or witnessed pesticide/fungicide use during the assignment, please complete the following USAID table in the greatest detail possible.

Agricultural Pesticide Application Details					Contact Details		
Date of Application	Pesticide/Fungicide Name	Application Rate	Crop/Pest	Location	Volunteer	Farmer	Applicator

PERSUAP Summary (if applicable) – To Be Completed by Field Staff
For Type 1 and Type 2 assignments, please write 2-3 sentences about how the Volunteer interacted with or witnessed pesticide use.
None of the pesticides were used during my observation.

Certification Declaration
I certify that the information contained in this document is true and accurate.

Dr. Alijar Cyrus Fouladkhah 4-30-2023

Signature of Volunteer, Date

_____

Verified and Approved by F2F Field Staff, Date

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Received and Reviewed by ACDI/VOCA Headquarters, Date