# Microbiome and Immunology

## Interactions for Risk Assessors from 21st Century Science

**SUNY ESF Environmental Risk Assessment (ENS 470)**

**Peg Coleman**

**March 28, 2023**

COLEMAN SCIENTIFIC CONSULTING

Peg Coleman

Microbial Risk Assessor

COLEMAN SCIENTIFIC CONSULTING

**Who we are**

- ESF alumna (Environmental Biology); ESF Alumni Board Member; consultant in **medical microbiology** and **microbial risk and benefit analysis**; selected as **Fellow of Society for Risk Analysis** (SRA) and elected to SRA Council

**What we provide**

- Analysis and training about safety of exposures to microbes in **air**, **foods**, **water**, and the **environment**

**Value of our services**

- **Enhance transparency** and give clients confidence to **separate facts** from **myths** about risk and health

http://www.colemanscientific.org/

# Microbiome and Immunology Lecture Outline
# Environmental Risk Assessment

1. Introduction to Human Superorganisms and Risk Analysis
   - Exposure Assessment
   - Dose-Response Assessment
   - Evidence Maps

2. Interconnections with Immunology
   – Microimmunosome
   – Colonization Resistance
   – Allergy and Inflammatory Diseases

3. Incorporating Microbiota into 21st Century Risk Analysis
   – Benefits AND Risks for Sustainable, Just, Regenerative Food Systems
   – Risk Analysis Quality

# SECTION 1: INTRODUCTION TO HUMAN SUPERORGANISMS AND RISK ANALYSIS

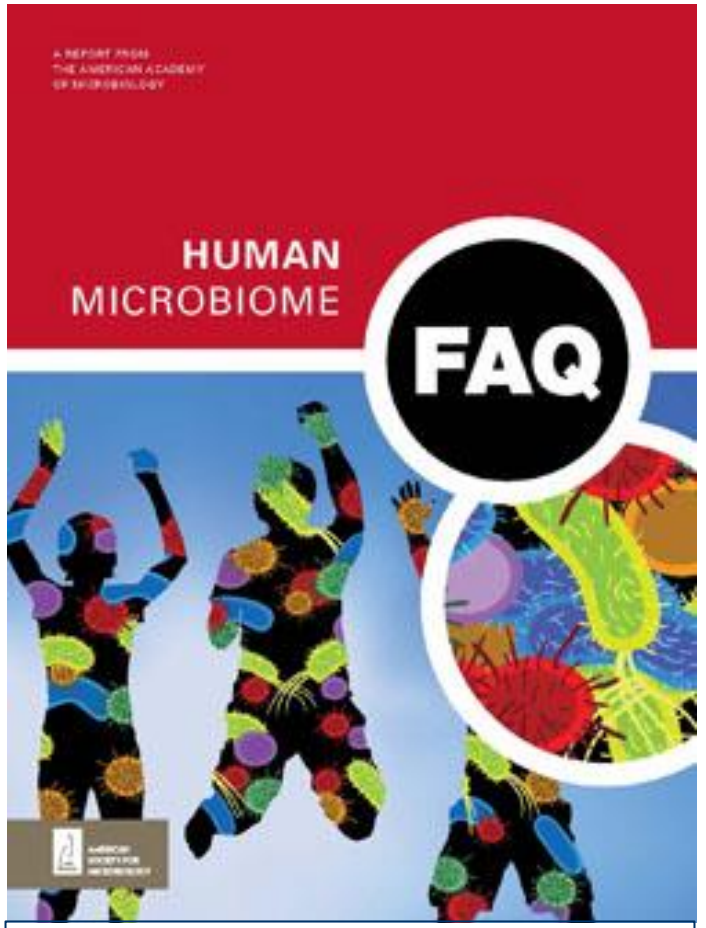
# Microbes and Microbiota

- Microorganisms share our environments: air, indoor and outdoor environments, soils, water, in/on bodies of **plants** and **animals**.

- Our relationships with microbes are **complex ecologically**.

- **Bacteria** may outnumber human cells by 3:1 ratio, and **viruses** may outnumber bacteria by 5:1 ratio! For every human gene, up to **300 non-human (microbial) genes**!

- Natural microbes in our bodies form dense, diverse communities, our **microbiomes,** that are now known to do more **good** than harm and **benefit** our health.



American Academy of Microbiology, 2014

- Studies on human **bacteriomes**, **viriomes** in health & disease expanding.

# Complex Relationships with Microbes:

## Presence Alone Insufficient to Predict Risk or Health

- **Probiotics:** Live microorganisms which, **when administered in adequate amounts**, confer a **health benefit** on the host (FAO/WHO, 2002)

- **Pathogens:** Live microorganisms which, **when administered in adequate amounts**, **cause disease** in the host

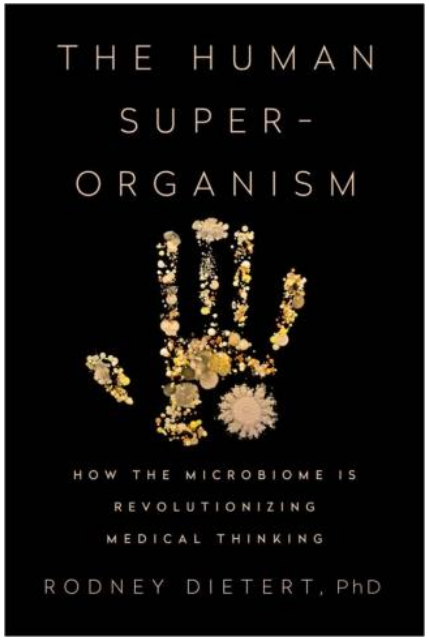
- **Commensals:** Live microorganisms which benefit by relationship with host but do not harm or provide known benefits to host
  - Commensal *Staphylococcus aureus* can become an opportunistic pathogen causing mastitis **at high doses**, e.g., above limit for toxin production (**100,000 bacteria per mL/g food**) controlled by **quorum sensing**

**Dose Matters for both Health and Disease**

# New Reality: Human Superorganisms!
# Sterility is NOT Healthful,
# Actually Harmful

**Emeritus Professor of Immunotoxicology Rodney Dietert** (Cornell University)
**2017 SRA webinar, *Protecting the Human Superorganism***

- ***Homo sapiens* + microbiota = human 'superorganism' (holobiont, 'supraorganism')**
- New medical landscape emerging in **21st century**, with **microbial ecology of superorganisms** challenging assumptions about health and disease, with emerging paradigm shift to '**managing our microbes**'




**Dietert, 2016**. The Human Superorganism: How the Microbiome is Revolutionizing Medical Thinking.

**Coleman et al., 2021.** Enhancing Human Superorganism Ecosystem Resilience by Holistically 'Managing our Microbes'.

# Some of My Best Friends are Germs

MICHAEL POLLAN

BOOKS | APPEARANCES | MEDIA | PRESS KIT | ARTICLES | NEWS | RESOURCES | ON TWITTER

**Some of My Best Friends Are Germs**

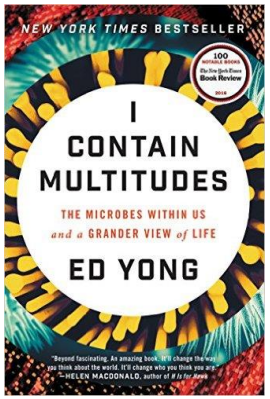
Michael Pollan
*The New York Times Magazine*, May 15, 2013

"Mother's milk, being the only mammalian food shaped by natural selection, is the Rosetta stone for all food," says Bruce German, a food scientist at the University of California, Davis, who researches milk. "And what it's telling us is that when natural selection creates a food, it is concerned not just with feeding the child but the child's gut bugs too."

Journalist Michael Pollan started thinking of himself as a 'superorganism' on March 7, 2013 when he received output from the citizen science project **American Gut Project**.

# Living in Microbial Ecosystems

- Genomic methods (e.g., Whole Genome Sequencing or WGS) challenge or falsify many assumptions of 20th century science




- Earth's ecosystems are full of **'superorganisms'** containing '**Multitudes'** of microbiota.


  - Human Genome Project began in 1990 and was completed 25 years ago (see https://unlockinglifescode.org/timeline?tid=4)
  - Human Microbiome Project began in 2007 and work is ongoing


- **Unified Microbiome Initiative** beginning in 2015 to study earth's diverse and connected microbial ecosystems

# Parallels in Chemical and Microbial Risk Assessment

- Similar framework elements
  - Hazard Identification
  - **Exposure Assessment**
  - **Dose-Response Assessment**
  - Risk Characterization
- For microbes, nearly all probabilistic **Quantitative Microbial Risk Assessment** or **QMRA**
- More extensive modeling of community interactions over time and space (Farm-to-Table Exposure Assessment)
- Exposure and Dose-Response Assessments adjusted for microbial **GROWTH** in ecosystems, foods, and hosts

# Chemical Risk Assessment with the Microbiome

OXFORD
SOT Society of Toxicology
www.toxsci.oxfordjournals.org

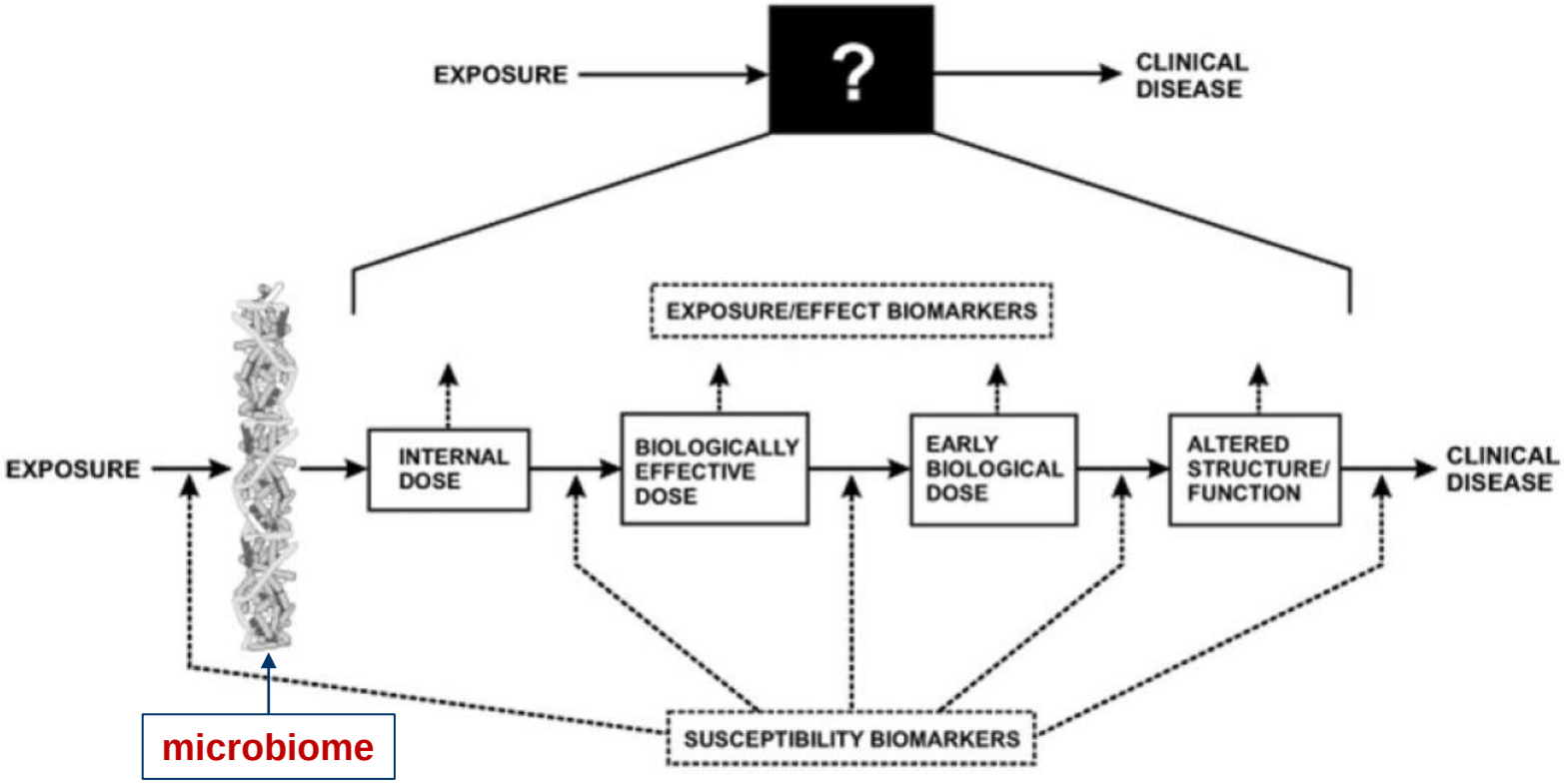
TOXICOLOGICAL SCIENCES, 2015, 1–9

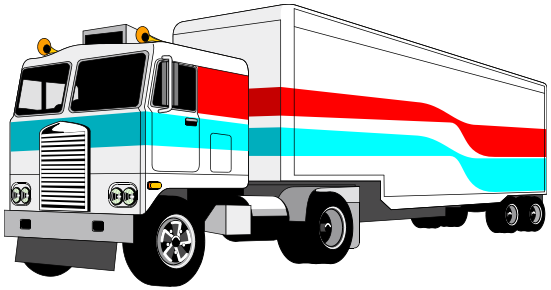
doi: 10.1093/toxsci/kfv013

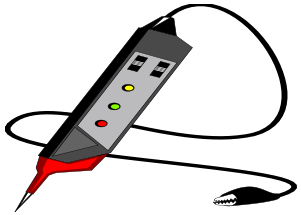
## Biomarkers for the 21st Century: Listening to the Microbiome

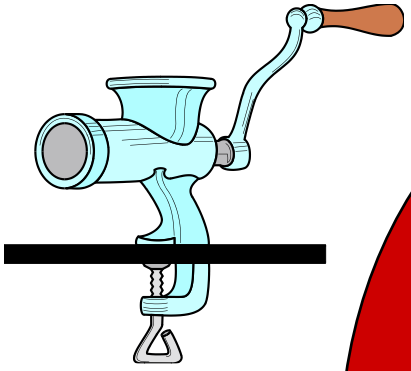
Rodney Reynolds Dietert* and Ellen Kovner Silbergeld†,1

*Cornell University College of Veterinary Medicine, C5 Veterinary Medical Center, Ithaca, New York 14853 and †Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland 21205

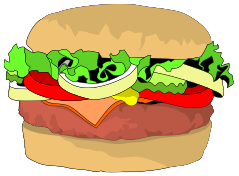


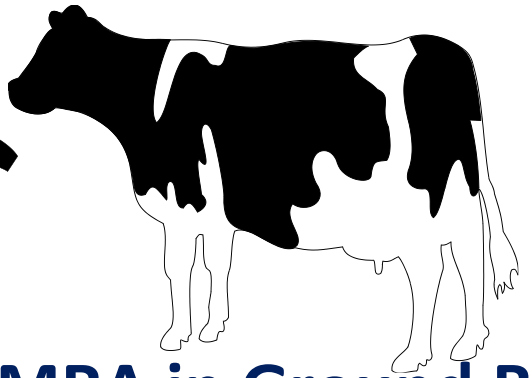






Exposure Assessment





Dose-Response Assessment

# STEC QMRA in Ground Beef (USDA 2001)

# Questions for Exposure Assessment

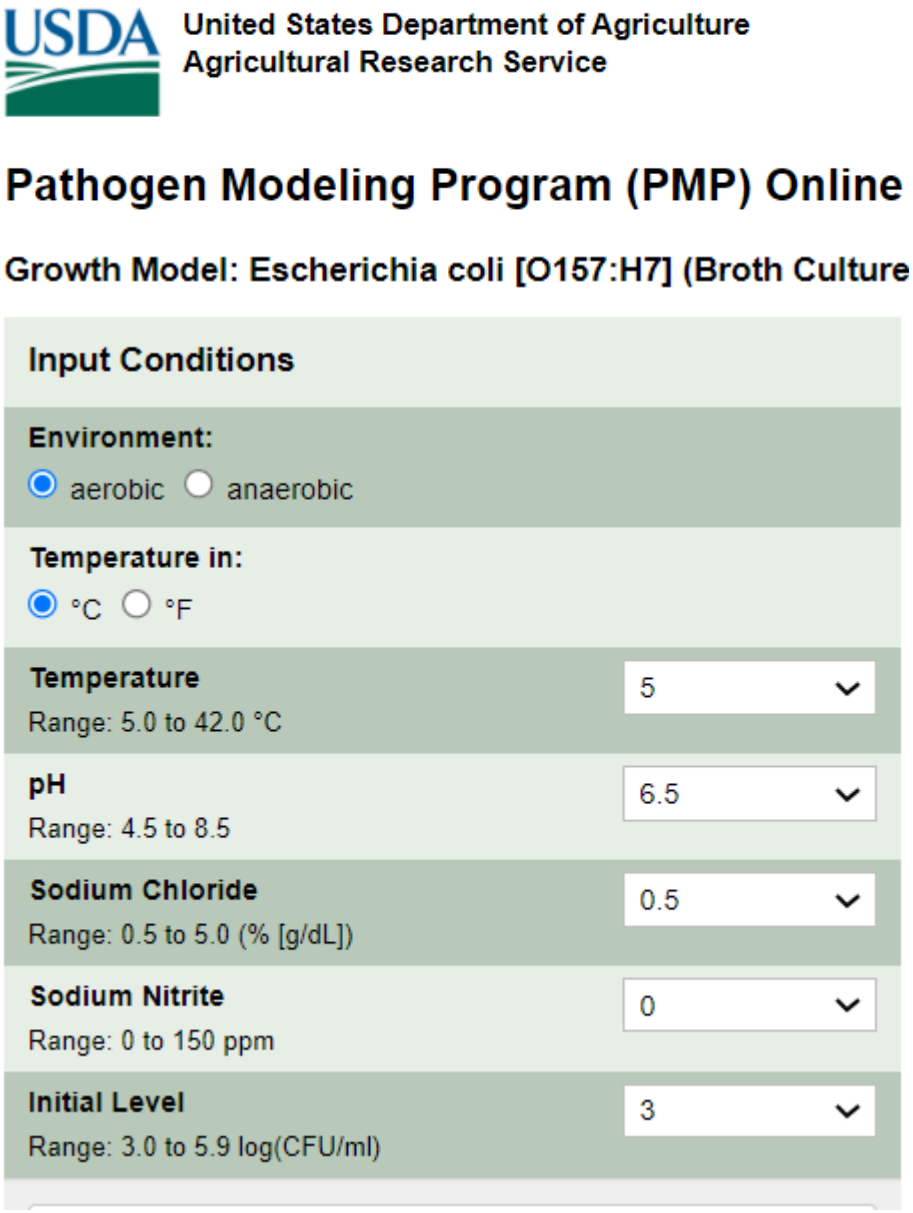
- Is a pathogen **detected** in serving of food?
- **How many** if detected?
  - Density (counts per serving) for positives
- Does pathogen **grow** (or survive) in food?
  - If yes, **how fast** (or how long)?
  - Depends on **temperature** AND **food microbiota**!

**Predictive Microbiology**

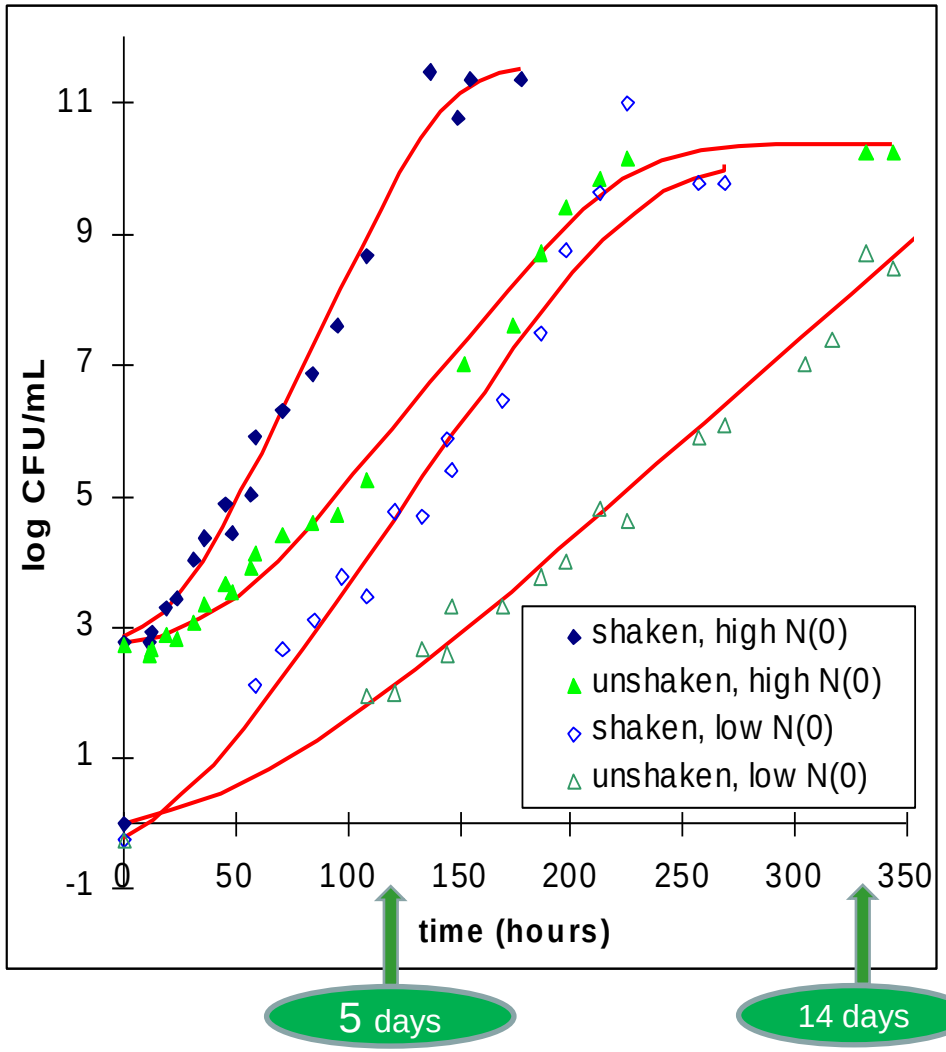
- **How many** pathogens (AND beneficial microbiota) are in **simulated serving** **(DOSE ingested)** at consumption?

# Early History of Predictive Microbiology

- Focus on factorial designs of **pure cultures** of cocktails of pathogen strains in **rich culture broths** lacking structure and natural microbes of raw foods
  - Temperature
  - pH
- **‘Worst-case’**, **‘fail-safe’** by design



# Growth at Sub-optimal Conditions




(Coleman et al., 2003)

## **STEC** (*E. coli* O157:H7)

- **Refrigeration temperature**
  **(upper limit for US survey, 50° F or 10° C; differences in growth at human body temperature or surface temperature in hot sun)**
- **Low initial counts**
  **(N0=1 bacterium/mL versus high counts N0=1,000 or more bacteria/mL)**
- **No shaking**
  **(like milk bottle in refrigerator versus culture flask on rotating shaker 24-7)**

# Exposure Assessment Issues for Foods

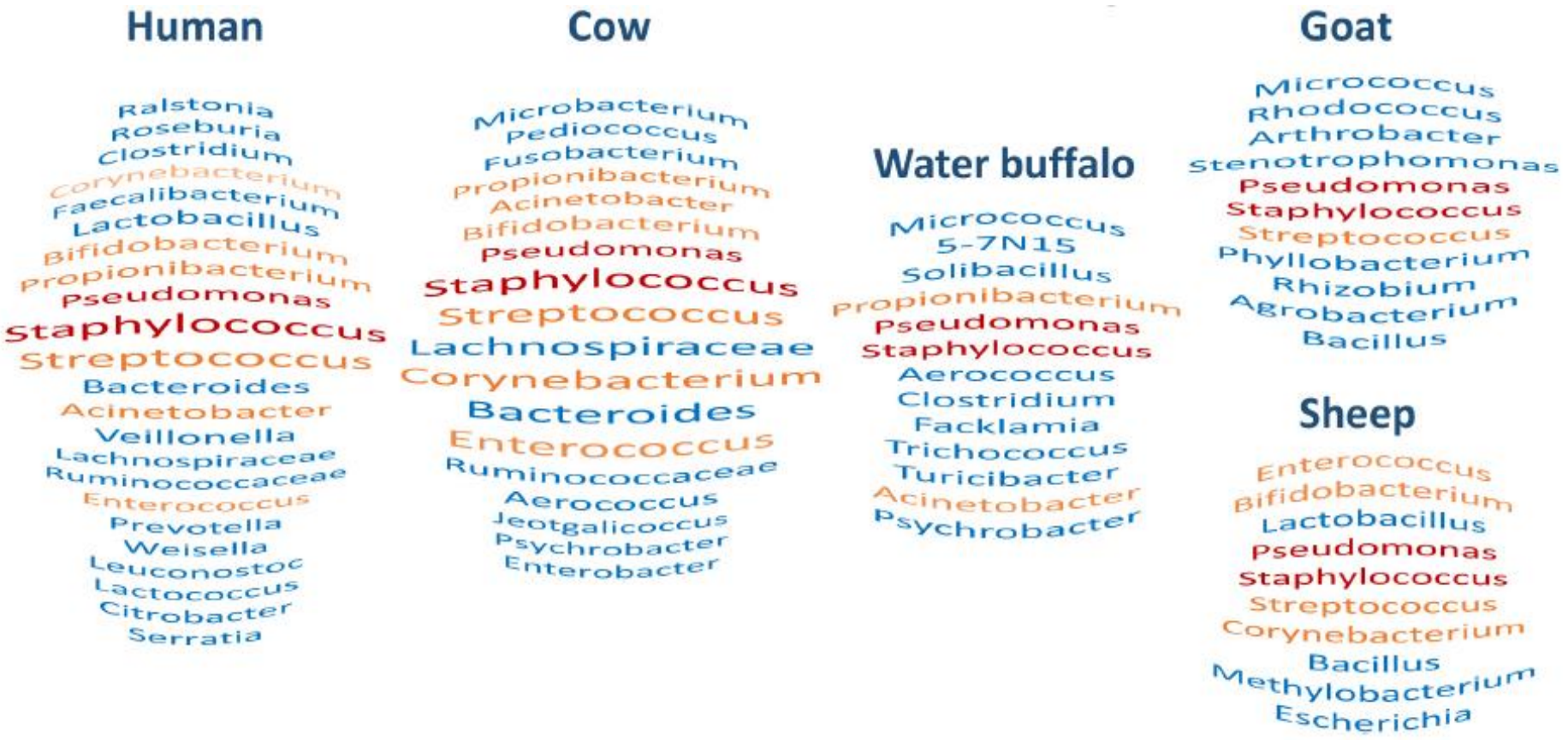
- **Optimal growth conditions** in laboratory experiments **unrealistic** for non-sterile foods (sub-optimal growth)
- Microbial growth depends on
  - How many **pathogens** present in foods (typically 1, 10, or <100, **not thousands or more**)
  - How many **competing microbes** present in foods (**tens of thousands or more in nonsterile foods**)
  - **Nature of food** (solid or unshaken liquids) and its **temperature**

**REALITY CHECK: growth models should adjust for realistic, sub-optimal conditions, including inhibitory effects of natural microbiota**

# Future of Predictive Microbiology in Raw Foods

- Model growth in **competition** with natural microbiota of raw, fermented foods and foods with starter cultures
- Estimate how many **pathogens** AND **beneficial microbiota** are in simulated serving of raw food **(DOSE ingested)** at consumption
- Determine if **benefits** exceed **risks**

# Dense and Diverse Natural Microbiota of Milks




**Oikonomou** et al., **2020**. Milk Microbiota: What Are We Exactly Talking About?
*Frontiers in Microbiology*

# Microbiota Out-Competes Pathogens

| Dairy Study | Numbers of Raw Milk Positives (range; mean; median) in CFU/mL | | | | |
|---|---|---|---|---|---|
| | **Standard Plate Count** | ***Listeria monocytogenes*** | **STEC/VTEC** | ***Salmonella spp.*** | ***Staphylococcus aureus*** |
| **D'Amico et al., 2008** Farmsted dairies N=62 | 62 | 3 | 0 | 0 | 17 |
| | (10 to $10^5$; **$4.9x10^4$**; 7.0 $x10^2$) | <1 | Non-detectable | Non-detectable | Unspecified; 250; <1 |
| | **Total Viable Count** | ***Listeria monocytogenes*** | **STEC/VTEC** | ***Salmonella spp.*** | ***Bacillus cereus*** |
| **Jackson et al., 2012** Commercial dairy silos N=184 | 184 | 23 | 30 | 5 - 33 | 4 |
| | $7x10^2$ to $5x10^5$ **$4.2 x10^4$** - | <0.006 to 29 0.65 0.12 | <0.006 to 1.1 0.19 0.26 | <0.006 to 60 0.75 0.12 | 3 to 93 0.75 0.12 |

Non-pathogen ***Pseudomonas*** dominates in both culture-dependent (SPCs; Tamplin 2002) and culture-independent (Liu et al., 2020) studies for multiple foods, grow optimally at low temperatures, outcompeting less adapted pathogens at refrigeration temperatures.

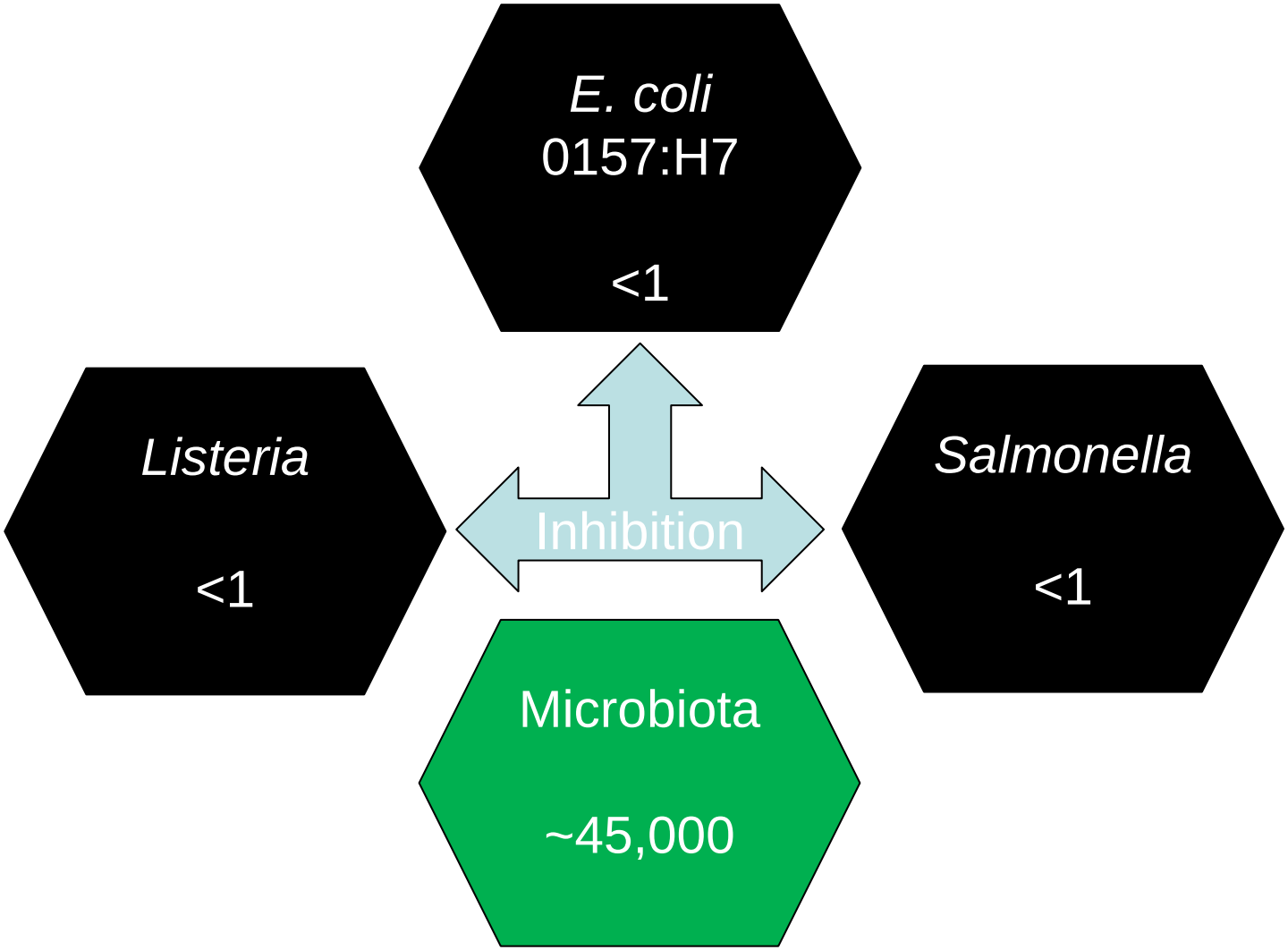
| Temperature (°F) | ***Pseudomonas* spp.** | ***Listeria monocytogenes*** | ***Escherichia coli* O157:H7** | ***Salmonella spp.*** | **Campylobacter spp.** |
|---|---|---|---|---|---|
| 36 | 0.09 | No Growth | No Growth | No Growth | No Growth |
| 39 | 0.11 | 0.01 | No Growth | No Growth | No Growth |
| 50 | 0.24 | 0.07 | 0.07 | 0.02 | No Growth |

Optimal growth rates of non-pathogen and pathogens (Coleman et al., 2003)

# Microbial Ecology

## Dominance of Milk Microbiota over Pathogens

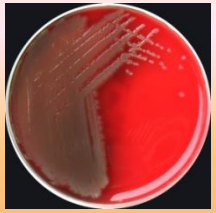
### (counts/mL when detected)



# Ecological Advantage:

## **Microbiota** Grows **Faster** than **Pathogens**

### *Pseudomonas*

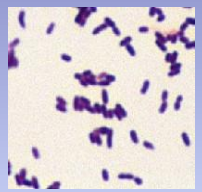


36° F 0.09/hr

39° F 0.11/hr

50° F 0.24/hr

### *Listeria monocytogenes*



36° F no growth

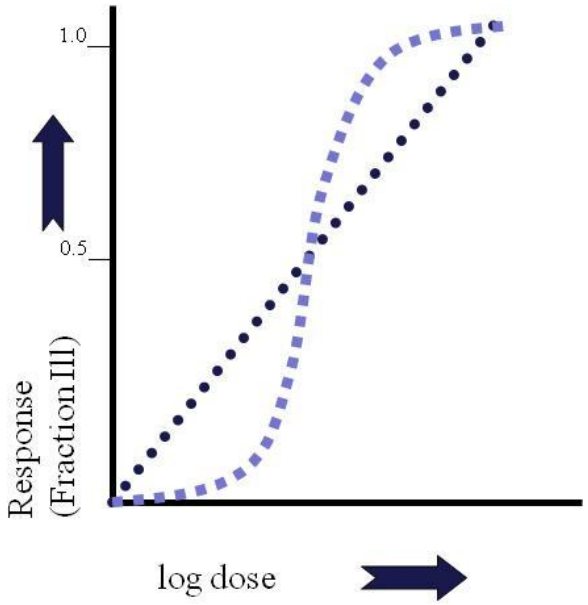
39° F 0.01/hr

50° F 0.07/hr

**Pseudomonads grow at the lowest temperature studied where pathogens studied do not grow at all**

# Assumptions and Science for Microbial Dose-Response Assessment

- **Increasing the pathogen dose generally increases**
  - Likelihood, severity, and duration of illness
- **Increasing the pathogen dose may decrease**
  - Incubation period, fraction with asymptomatic illness, time to morbidity or mortality




- **Exposure ≠ illness (or mortality!)**
  - Healthy superorganism defends against many pathogen exposures (including COVID!)
- **Low doses may not cause illness**
  - Innate defenses (including **gut microbiota** exerting **colonization resistance**) prevent adherence and growth of low doses of pathogens
- **Low-dose linearity and no threshold assumptions not feasible**

Exposures even for feared pathogens (e.g., *E. coli* O157:H7) asymptomatic for farm families including children & healthy positive 6-month old baby (Wilson et al., 1996; Karmali et al., 1996; Haack et al., 2003)

# Evidence for Thresholds for Human Illness

Healthy people have **innate resistance** to many pathogens **particularly at low doses**.

**Salmonellosis** cases observed at doses greater than $10^9$ or **1,000,000,000** ingested bacteria for *Salmonella* Pullorum

Coleman & Marks, 2000; Coleman et al., 2017

**Listeriosis** cases not simulated at doses less than $10^4$ or **4,000** ingested bacteria for *Listeria monocytogenes*

FDA, 2008

**Tularemia** cases observed at doses greater than $10^6$ or **1 million** ingested bacteria (*Francisella tularensis*)

Thran, 2015

# Traditional Dose-Response Assessment for QMRA

## Pathogen

- Characterize doses causing no response, asymptomatic infection, illness, or fatalities

## Host

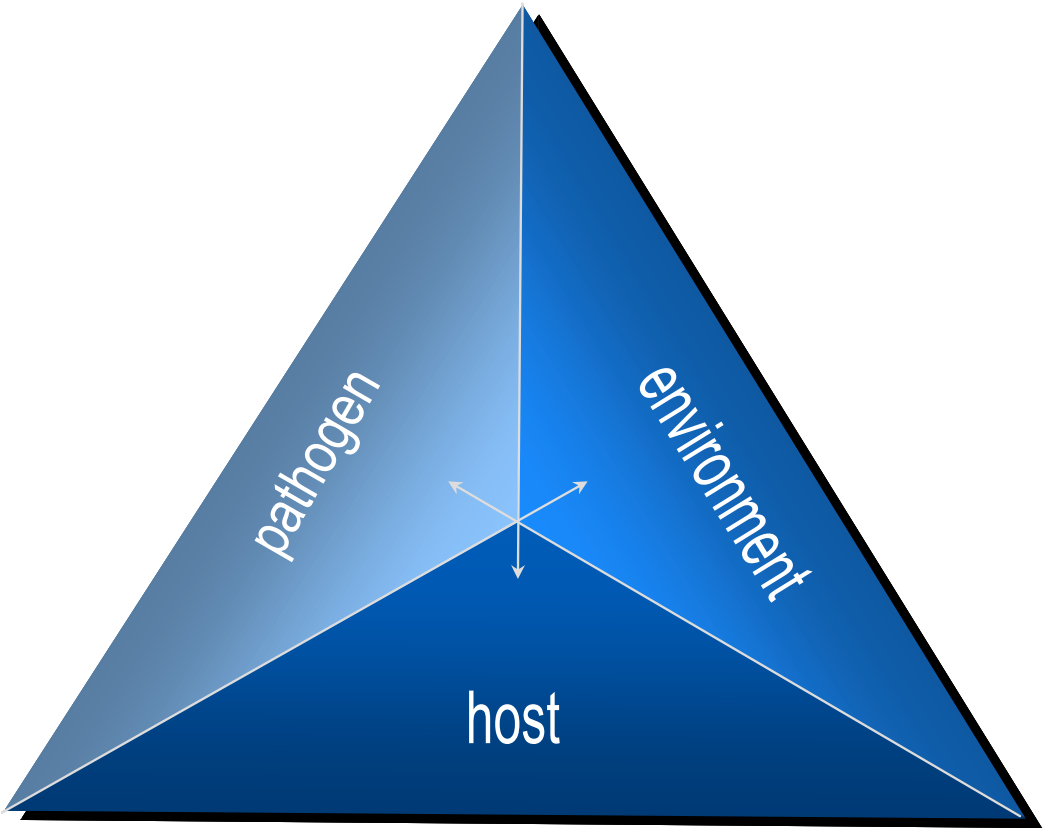
- Characterize dose-response relationships for populations at risk

## Environment

- Characterize conditions causing disease

## Interactions

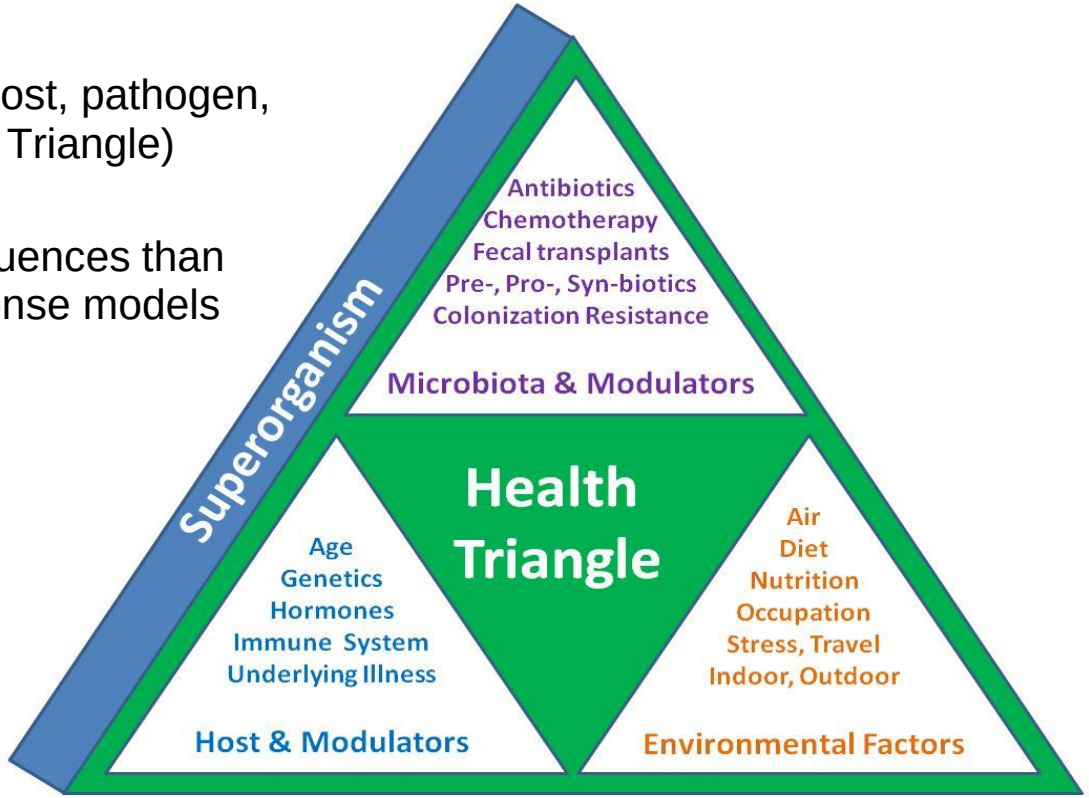
- Characterize conditions favoring sporadic disease and outbreaks




**Disease Triangle**

# Evolution of Dose-Response Assessment in 21st Century

- Acknowledgement of ecosystem effects, superorganism and modulators
- More complex than interactions of host, pathogen, and environmental factors (Disease Triangle)
- Wider context for environmental influences than considered for microbial dose-response models
  - Age
  - Diet
  - Drugs
  - Exercise
  - Immune status
  - Indoor and outdoor environments
  - Occupation
  - Stress
  - Travel



# Evidence Map Approach to Promote Transparency for Evidence on the 'State of Science'

**Evidence Maps: Communicating Risk Assessments in Societal Controversies: The Case of Engineered Nanoparticles**

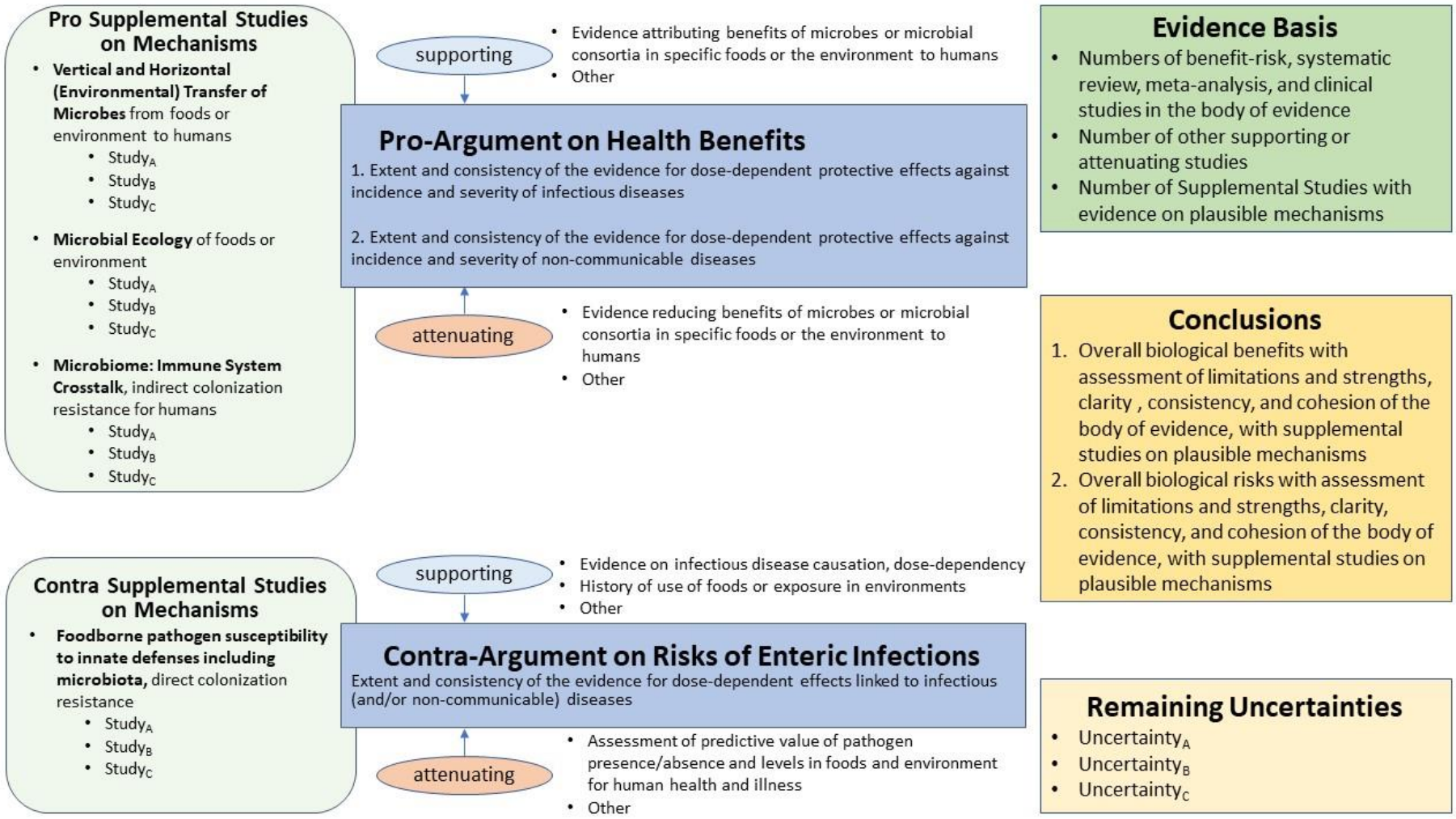
- Promote **openness** and **transparency** for evaluating ambiguous and conflicting scientific evidence for applications in risk analysis
- Provide **structured graphical representation** of the **evidence basis**, drawing attention to both **pro-** and **contra-** arguments, with **supporting** and **attenuating** data
- Assist **risk analysts** in **avoiding 'traps** such as **confirmation bias'** that may **distort** judgments about **weighing and synthesizing evidence** from **multiple disciplines**
- Facilitate **constructive dialogue** between diverse perspectives/opinions of all **stakeholders**, including decisions makers and educated public
- Assist **diverse experts** and **non-experts** to acknowledge the full body of scientific evidence, the **evidence basis**, as well as **quality of evidence** and **uncertainty**



**Wiedemann** et al., **2011**. Evidence Maps: Communicating Risk Assessments in Societal Controversies: The Case of Engineered Nanoparticles. *Risk Analysis*

# Evidence Map Template

Coleman et al., 2021a. Enhancing Human Superorganism Ecosystem Resilience by Holistically 'Managing our Microbes' (motivated by Wiedemann et al., 2011)



# ‘Managing our Microbes’ for Health and Resilience

## ➢Introduce commensal competitors to pathogens (*C. difficile)*

- **Fecal Microbiome Transplant** (FMT) from healthy hosts to restore colonization resistance, health, and resilience of gut ecosystem (**Durham** et al., **2020**)

- ***Clostridium scindens*** (**Parkar** et al., **2021**) or other probiotics as biological detoxification tools to suppress *C. difficile* germination and toxic effects, **increase microbiome diversity** and **gut** and **immune health**

## ➢Modify gut ecosystem functionality and integrity

- Enhance **butyrate-producing microbiota** to enhance protection against GI disease (**Neelis** et al., **2020**) and **respiratory viral infections** (**Haak** et al., **2018**)

## ➢Modify diet to increase gut diversity and richness and shift commensal-pathogen competition for nutrients

- **High-fiber diets** and **prebiotics** increased production of secondary bile acids and/or SCFAs and reduced **obesity** and metabolic diseases including **type 2 diabetes** (**Zhang** et al., **2020**)

# Section 1 Summary

1. 21st Century Science: ***Homo sapiens* plus** the dense, diverse microbial communities of our **microbiomes** function as '**superorganisms**' or holobionts
    - Microbes our **partners** in **health** and **disease**

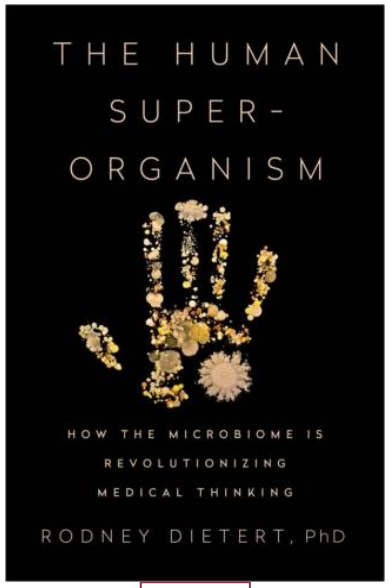
2. Ecosystem dynamics include **succession**: 'core' gut microbiota may begin developing *in utero* and continues though major life stages, with microbial inputs from air, food, and water necessary for health

3. Microbiota of **ecosystems**, human **gut**, and **foods** are **interrelated**; contribute to **health** (and **disease**)

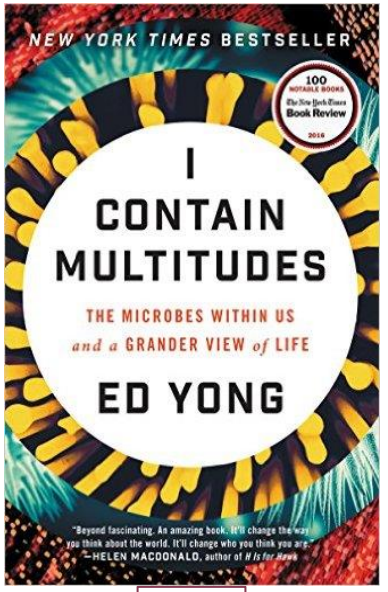
# SECTION 2: INTERACTIONS WITH IMMUNOLOGY

# More on Immunology in 21st Century

- **Classical portrait**
  - **Surveillance** and **destruction** of pathogens
- **Expanded portrait with emerging insights for 'superorganisms'**
  - '**Microimmunosome**' includes dense and diverse microbiota that **synergistically and cooperatively protects against pathogens**
  - **Joint management of homeostasis** with cross-talk between resident microbes and host immune cells, particularly at mucosal epithelia
    - Thousands of commensals (Firmicutes, Bacteriodetes, Actinobacteria, Proteobacteria phyla) contribute to mucosal immune homeostasis in the gut
  - **Alliances** change with context
    - commensals can express mutualism or pathogenicity under certain conditions



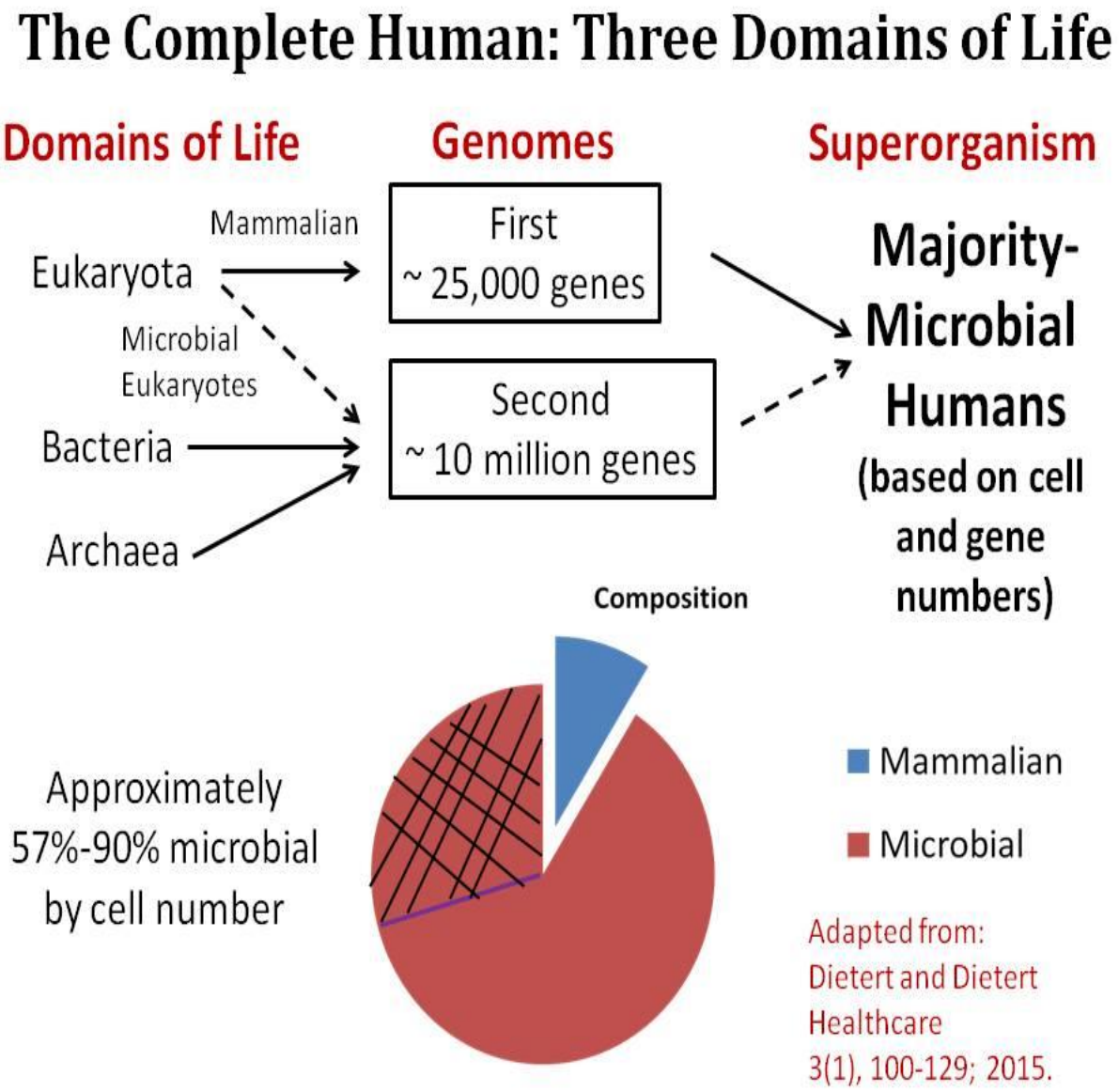

2016




2016

# Learnings on Human Superorganism

Rod Dietert, 2017 SRA webinar



# Learnings on Human Superorganism

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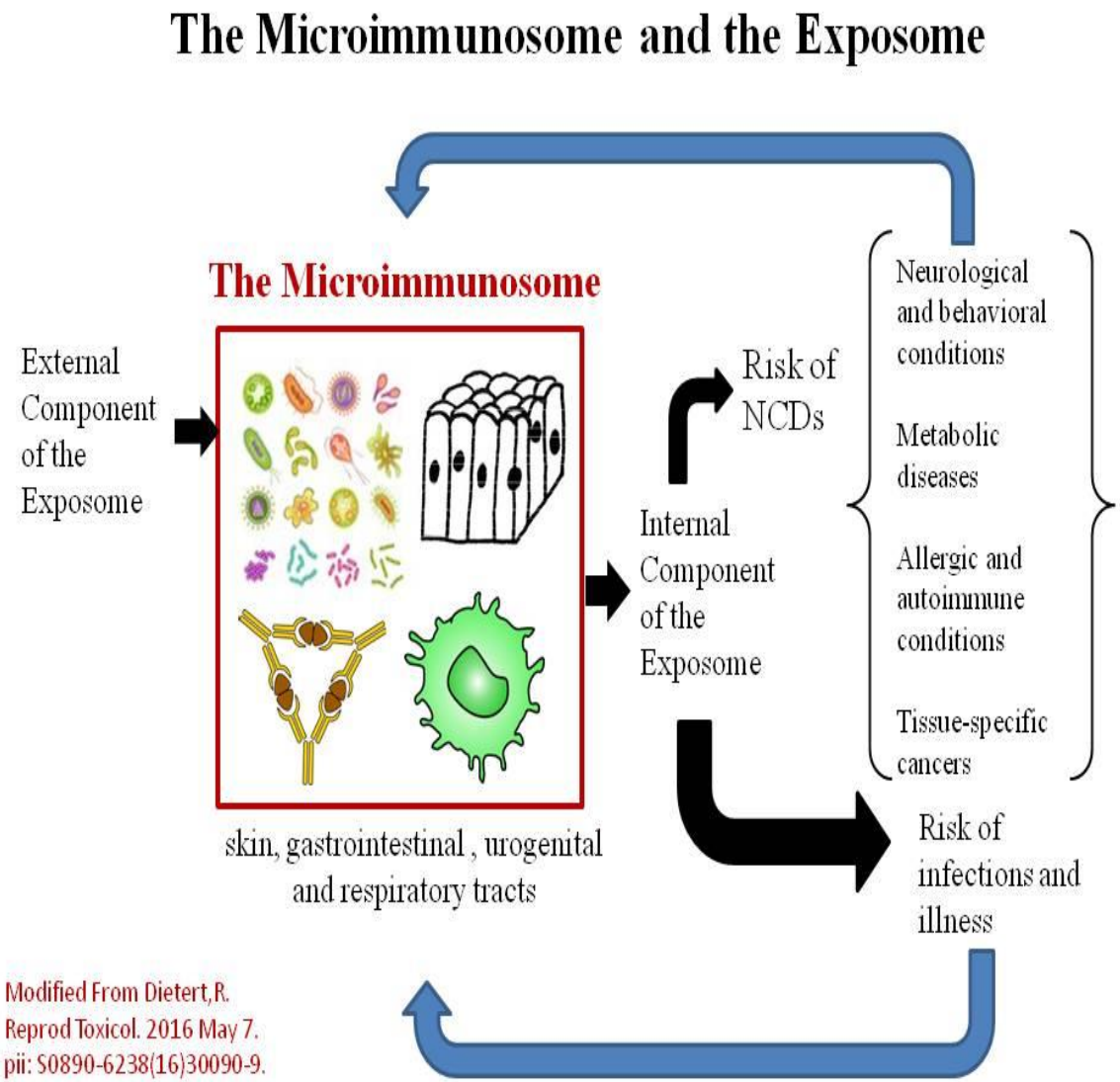


# Immunology in Updated Glossary for Risk Assessors

(Coleman et al., 2018)

- ***Adaptive (acquired) immune system*: Host defenses produced in response to invasion** by specific infectious agents involving humoral immunity with **antibodies** formed by B-lymphocytes and cell-mediated immunity through T-lymphocytes and activated macrophages.

- ***Innate immune system*: Host defenses always present** and effective against low doses of most infectious agents, including: **physical barriers** (e.g., skin and mucous membranes, intestinal barrier function); **complement** and other proteins that mark invaders for phagocytic removal; **natural killer cells**; **phagocytic cells** (macrophages and monocytes, neutrophils); pattern recognition proteins including **Toll Like Receptors** that bind pathogen-/microbe-associated molecular patterns (flagellin, peptidoglycans, lipopolysaccharides) for removal/tolerance; and **washing and enzymatic actions of bodily secretions** (e.g., tears, saliva, gastric juice, bile).

  High doses of pathogens can overwhelm the innate immune system and cause disease in healthy and dysbiotic hosts.

# Dysbiosis

- Refers to **microbial imbalance** resulting from a change in the number or types of bacteria on or inside the body.

- Is most prominent in the **digestive tract** or on the **skin**, but can also occur on any exposed surface or mucous membrane.

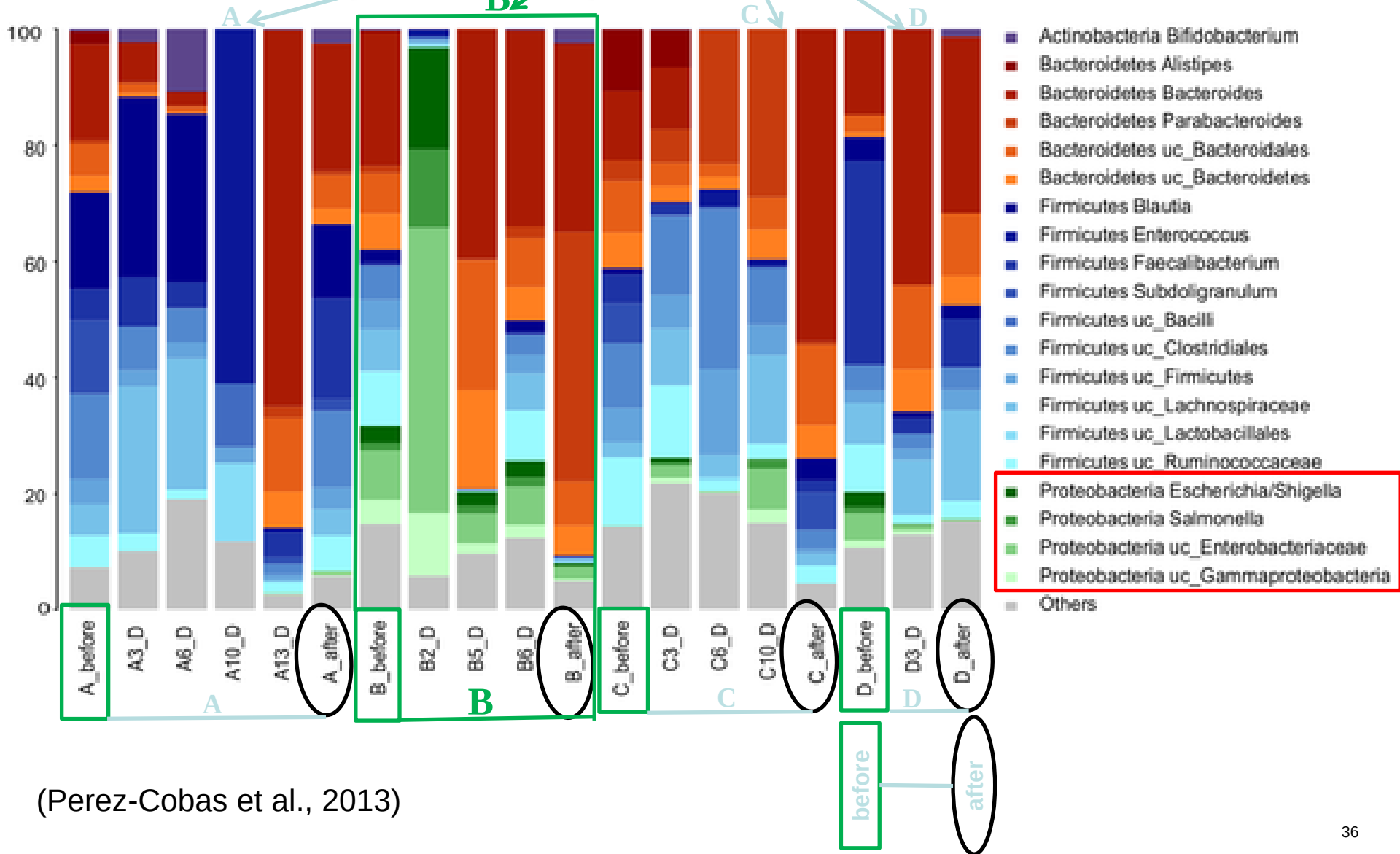
- May have a role in illnesses such as inflammatory bowel disease, chronic fatigue syndrome, obesity, or certain cancers. One cause of dysbiosis is **antibiotic treatment**.

(Glossary of the Gut Microbiome Compiled by The American College of Gastroenterology World Digestive Health Day | May 29, 2014)

# Antibiotics Shift Gut Microbiota in Four Volunteers

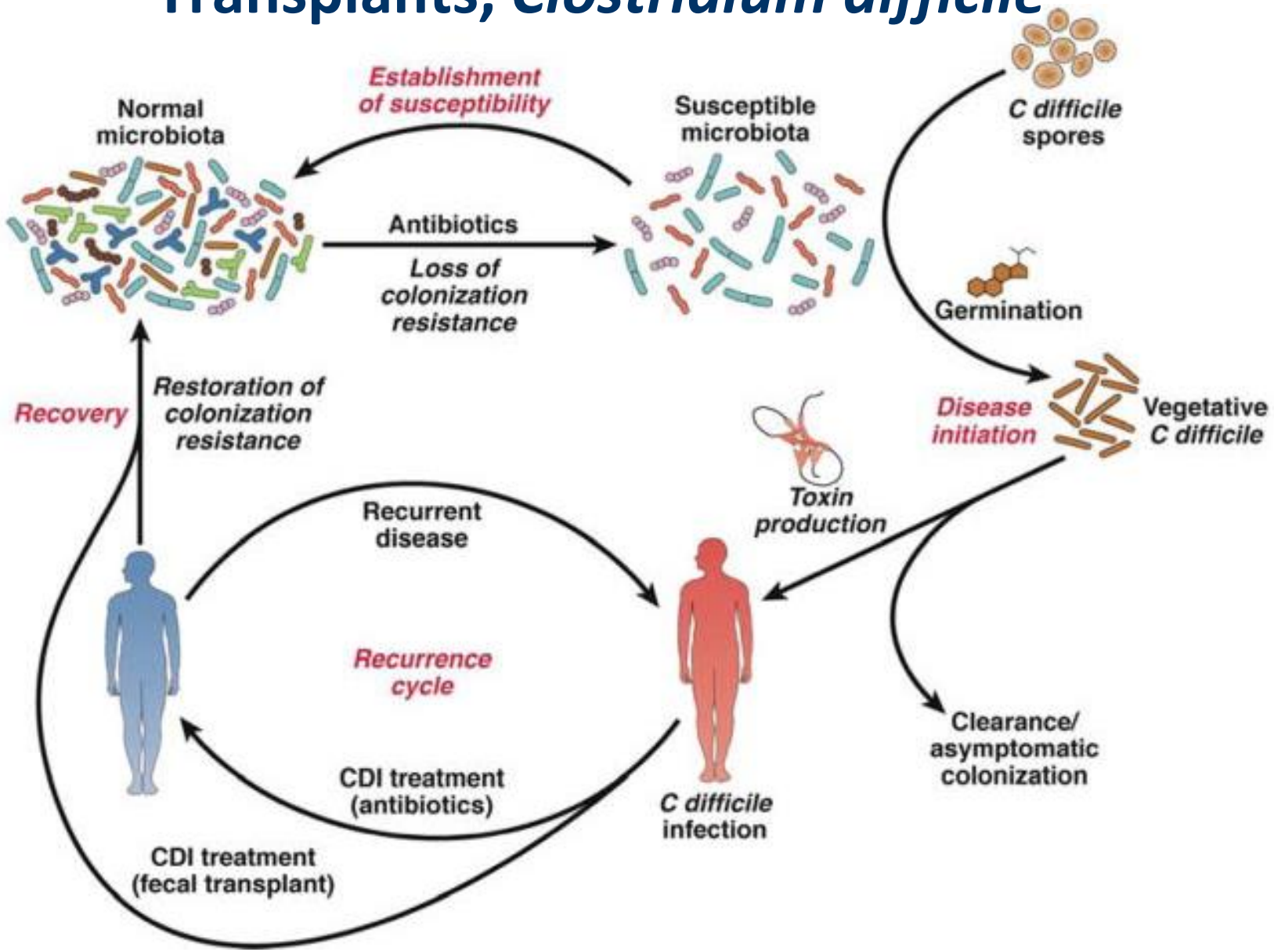
**DYSBIOSIS**

**disruption of the indigenous gut microbiota; factors include antibiotic administration, infection, malnutrition, stress, changes in diet, and travel**



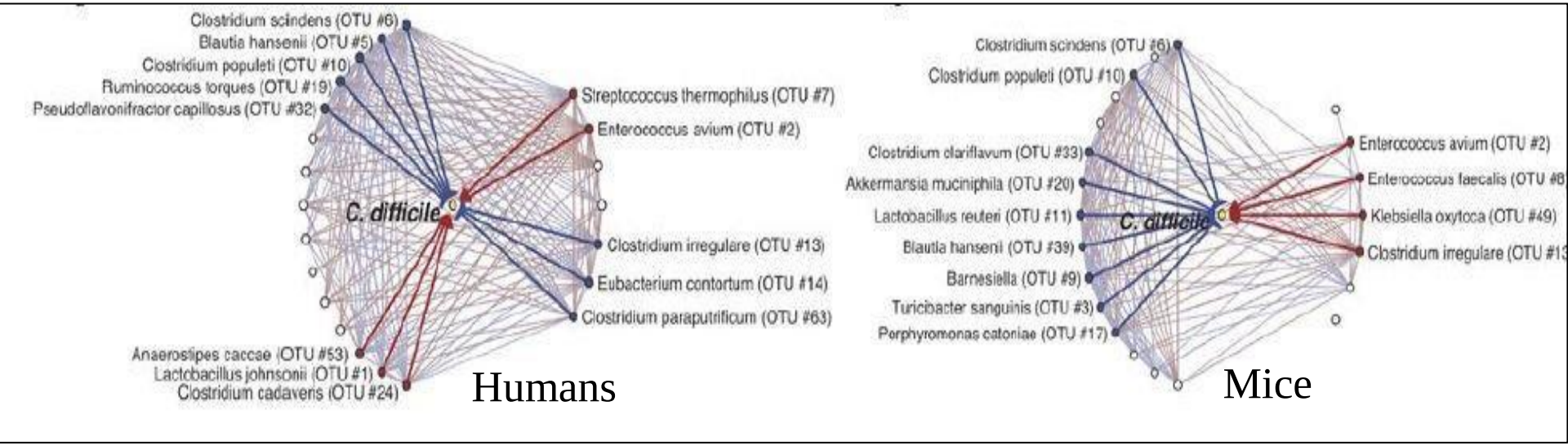

(Perez-Cobas et al., 2013)

# Colonization Resistance, Fecal Microbiome Transplants, *Clostridium difficile*




Britton and Young Gastroenterology, 2014; Dietert, 2017, 2018

# Microbes Promoting Resistance or Susceptibility to *C. difficile*




Similar patterns from inference modelling of subnetworks of metageomic data.

- **Blue lines** mark **resident** microbiota predicted to inhibit *C. difficile* growth blooms in **healthy hosts.**

- **Red lines** mark **dysbiotic microbiota** predicted to promote *C. difficile* growth blooms in **immunocompromised hosts**.

Buffie et al., 2015

# Opportunistic Pathogens

can cause **nosocomial** (hospital-acquired) infections, serious infections in neonates and immunocompromised people, and those on ventilators and other medical devices, with wounds, and with **antibiotic-disrupted microbiomes**.

Example:

Microbiota disruptions create niche for ***Clostridium difficile*** that can be detected by trained noses

Cliff the *C. difficile* detection dog

http://www.dailymail.co.uk/health/article-2247688/Meet-Cliff-remarkable-super-sniffing-dog-detects-hospital-superbugs.html

See: Bomers et al. A detection dog to identify patients with Clostridium difficile infection during a hospital outbreak. J Infect. 2014 Nov;69(5):456-61.

# Ecological Concepts in Updated Glossary for Risk Assessors


*Evolution Fueling 'Microbiome Revolution'*

- antibiotic-induced susceptibility in mice treated prior to challenge with doses of *Salmonella* (Bohnhoff et al., 1954; Endt et al., 2010)

- ***colonization resistance*** – protection of hosts with healthy microflora/microbiota against pathogens, with dose- and time-dependencies (Van der Waaij et al., 1971; Brugiroux et al., 2016)

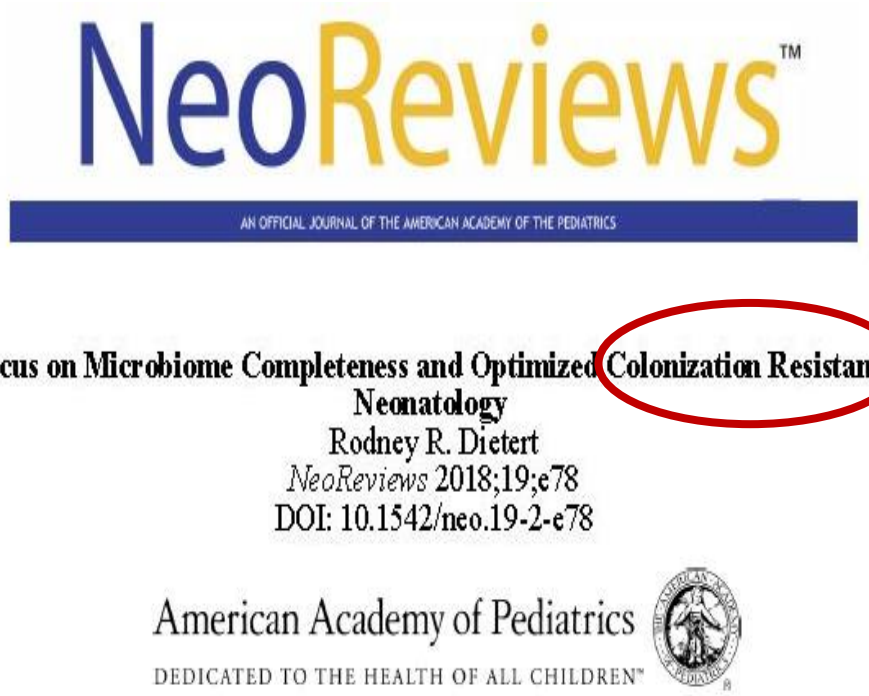
- Human Microbiome Project and Unified Microbiome Initiative beginning in 2007 and 2015, respectively, to study earth's diverse and connected microbial ecosystems

- ***superorganism*** – a hybrid consortium of human and microbial communities that together, synergistically and cooperatively, regulate health and disease (Turnbaugh et al., 2007; Dietert, 2016)

**From Coleman et al, October 2018 issue of *Risk Analysis***

# Colonization Resistance

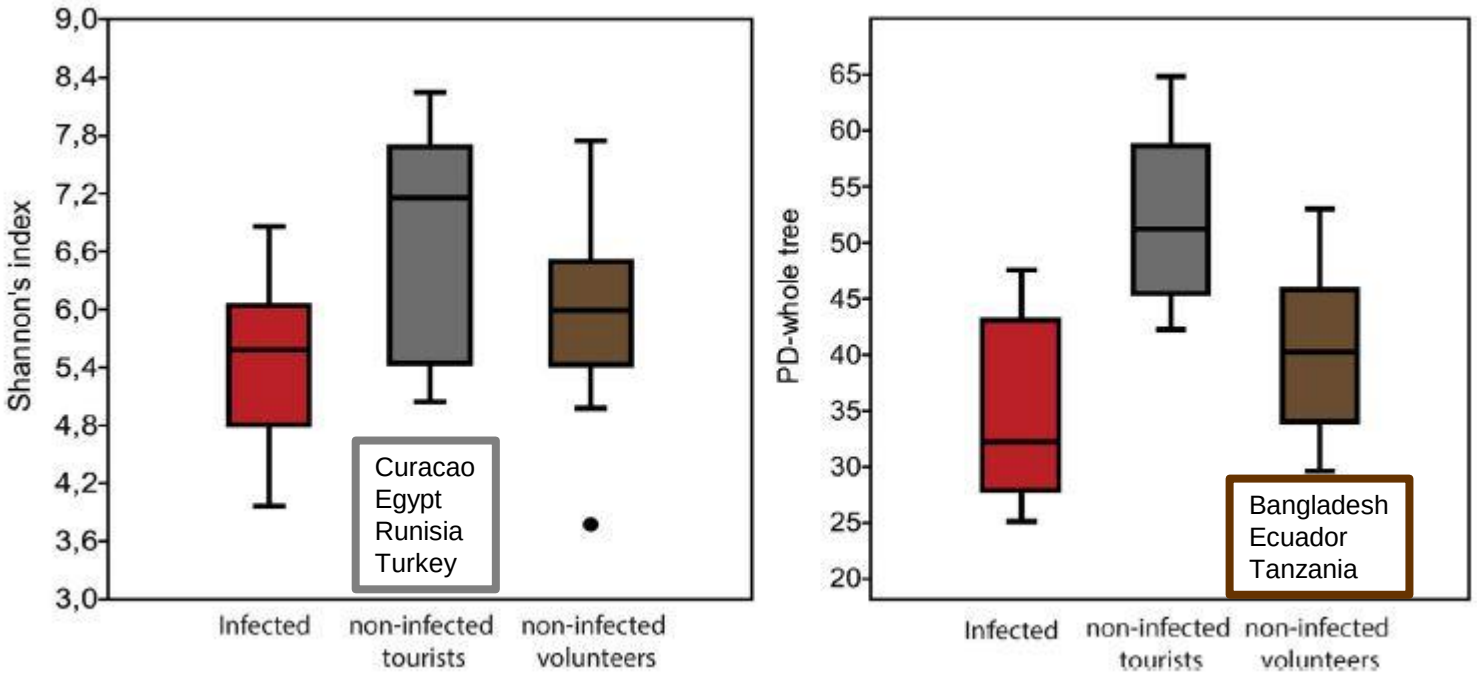
- Microbiota of healthy people can effectively **inhibit colonization** and **overgrowth** by invading pathogens.
- First observed in 1954 and termed **colonization resistance** in 1971, current methods of the 21st century are revealing mechanisms.
  - associated with a **stable and diverse gut microbiota** that do not trigger inflammation (**homeostatis**)
  - involves specific interactions between the **immune system** and the **microbiota**




Bohnhoff et al.,1954; Van der Waaij et al.,1971; Barza et al.,1987; Lawley & Walker, 2013; Newton et al., 2013; Gahan and Hill, 2014; Pham and Lawley, 2014; Malys et al., 2015; Perez-Cobas et al., 2015; Sassone-Corsi and Raffatellu, 2015; Sassone-Corsi and Raffatellu, 2015; Stecher, 2015; Brugiroux et al., 2016; Zipperer et al., 2016; Isaac et al., 2017

# High Microbiota Diversity, High Resistance

## Colonization Resistance to *Campylobacter*




Healthy tourists 18-64 years of age who traveled in groups from Sweden and acquired travelers' diarrhea

**Composition of human faecal microbiota in resistance to *Campylobacter* infection**

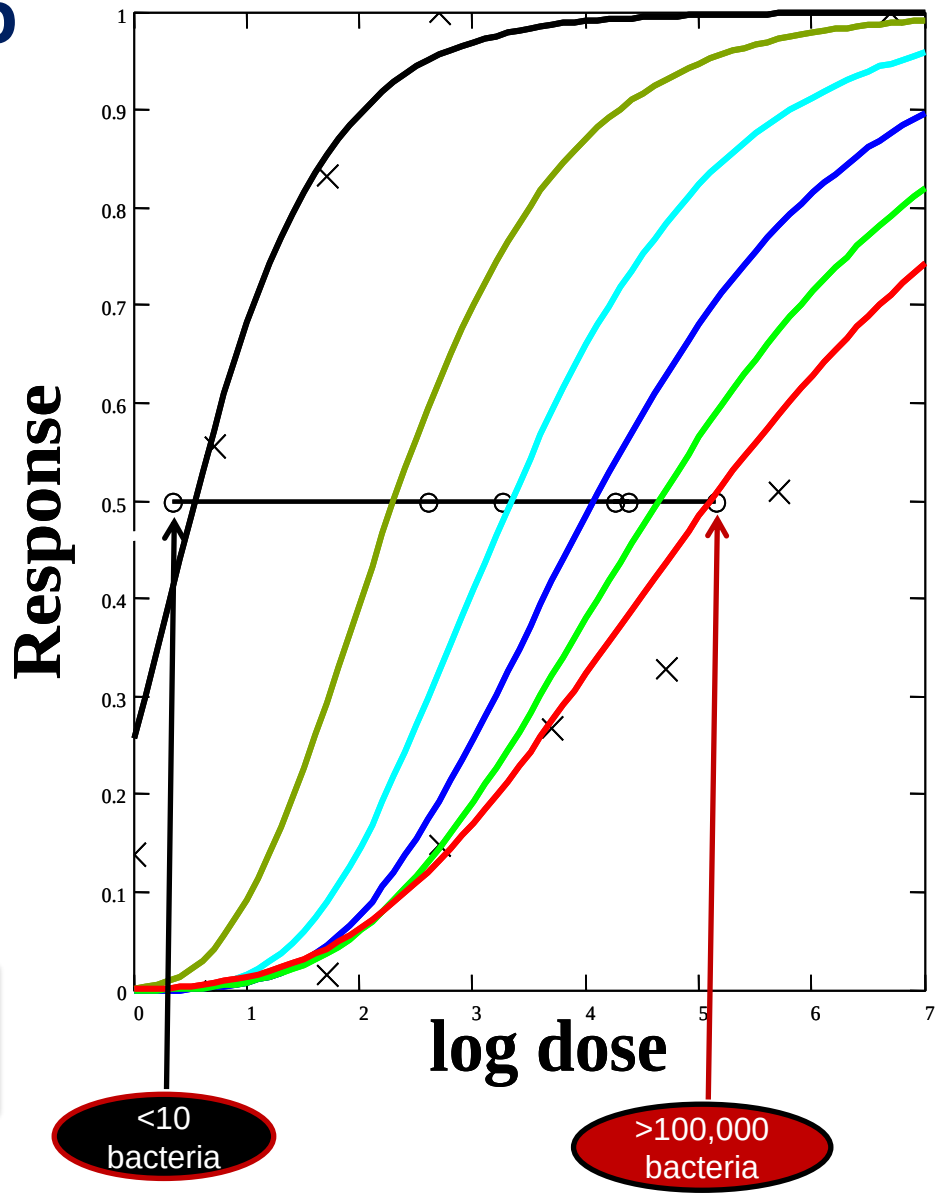
C. Kampmann[1,2,3], J. Dicksved[2,4], L. Engstrand[5,6] and H. Rautelin[2,7]

**2016**

# Colonization Resistance to Salmonellosis in Mice

- **Normal** animal **challenges** with increasing doses of *Salmonella enteritidis* (**red line**)
- **Antibiotic** 1 day before challenge disrupts colonization resistance and increases susceptibility (**black line**)
- **Microbiota recovers** within 5 days (**bright green line**) to normal magnitude of colonization resistance

**Host susceptibility increases five orders of magnitude!**

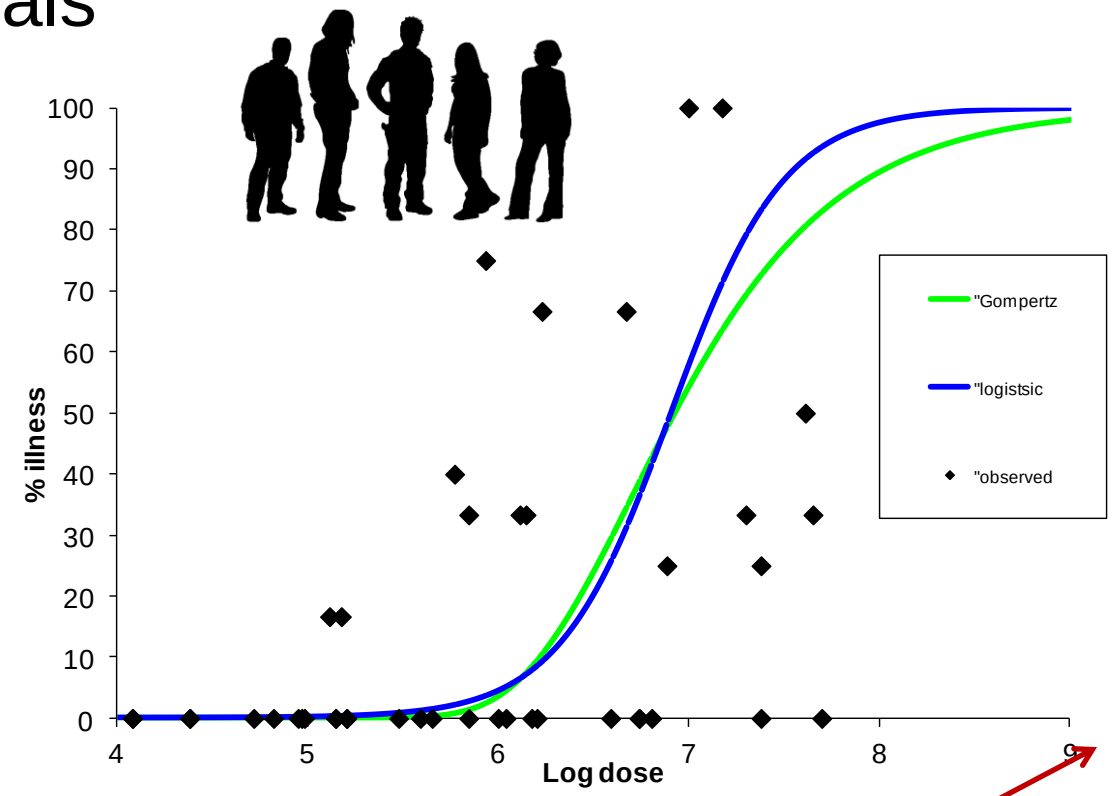



(Coleman and Marks,1999, 2000; Coleman et al., 2017)

# *Salmonella* Strains Administered to Humans

Human clinical trials

- anatum
- bareilly
- derby
- meleagridis
- newport

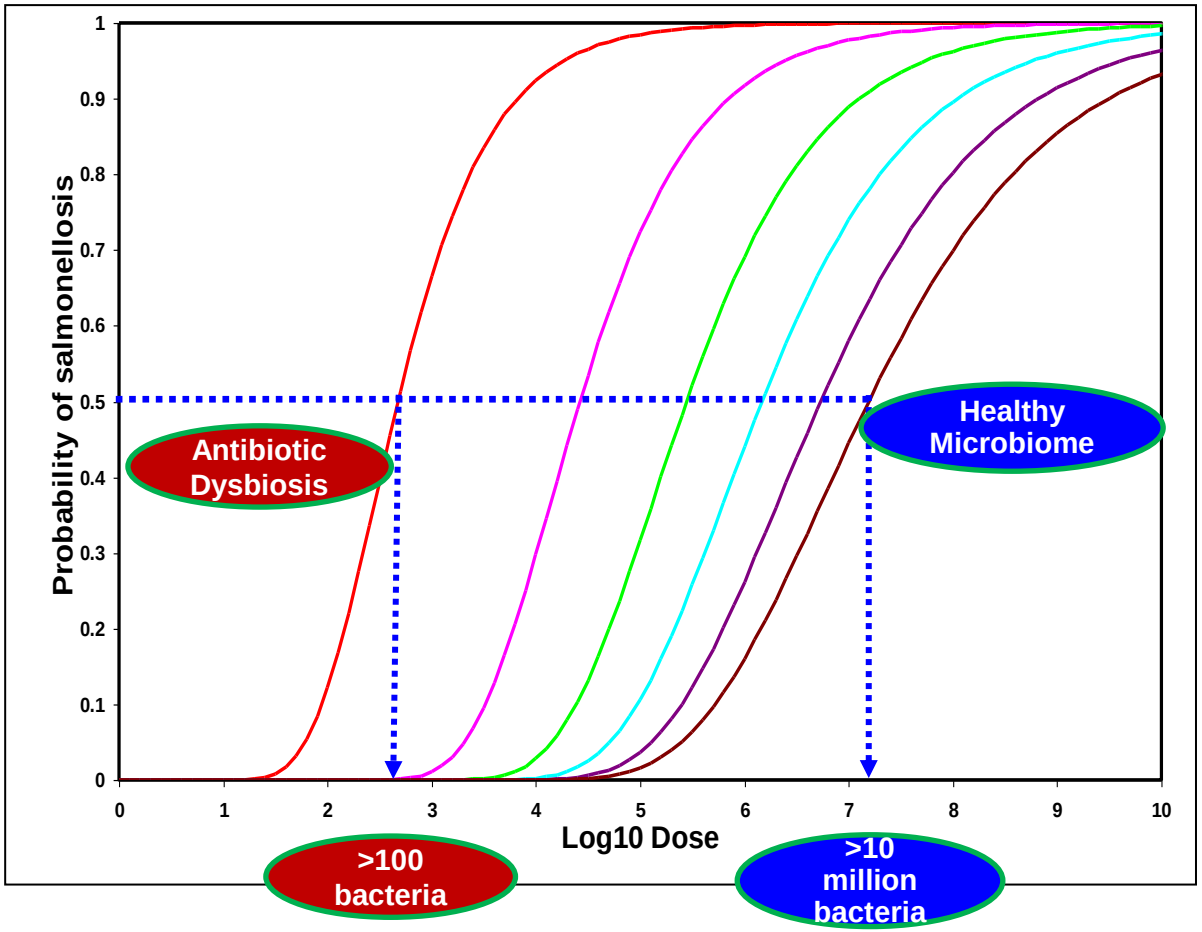



- **pullorum** (statistically significant threshold at $10^9$ bacteria)

(Coleman and Marks, 1998; Coleman et al., 2017)

# Increased Susceptibility for Antibiotic-Induced Loss of Colonization Resistance

- Half of healthy volunteers ill after dosing with ~$10^7$ (ten million) *Salmonella* bacteria (**brown line**)
- Half of volunteers with antibiotic dysbiosis likely ill after dosing with ~$10^2$ (>100) (**red line**)
- Microbiota recovers over time (2 days, pink line; 3 days, green, 4 days, aqua line, 5 days navy line)
- Indirect evidence of $10^5$ magnitude of **colonization resistance** (mouse and human data)




(Coleman and Marks, 1999; Coleman et al., 2017, 2018)

# Collaborative Manuscript with Cornell Emeritus Professor of Immunotoxicology Rodney Dietert

*applied microbiology*

an Open Access Journal by MDPI

## Enhancing Human Superorganism Ecosystem Resilience by Holistically 'Managing Our Microbes'

Margaret E. Coleman; Rodney R. Dietert; D. Warner North; Michele M. Stephenson

*Appl. Microbiol.* **2021**, Volume 1, Issue 3, 471-497

- **Disappearing Dogmas of 20th Century Biology and Medicine**
- **Immune Integrity, Balance, and Regulation of Inflammation Influenced by Microbiota**

| 20th Century Dogma | Update from 'Microbiome Revolution' |
|---|---|
| **The newborn is fully prepared immunologically to fight all diseases.** | The newborn immune system must postnatally co-mature with the microbiome to be immunologically balanced and fully prepared to fight diseases. When this fails to happen, infectious, autoimmune, inflammatory, and allergic diseases are more likely to occur beginning in childhood and extending throughout the lifespan. |
| **Barriers defining species are driven by host chromosomal genes (hybrids lethal or sterile).** | Immune-microbiome incompatibility can also determine species barriers (antibiotic destruction of the microbiome can remove some species barriers). This highlights the almost sacred biological bond that has existed for millennia between the immune system and friendly microbiota. They are inseparable from both an evolutionary and a systems biology perspective. |
| **Most microbes are a threat to human health.** | Many microbes are required for human health. Essential functions include: i) colonization resistance; ii) production of vitamins and neurochemicals; iii) metabolism of food, chemicals, drugs, and bile acids; iv) maintenance of epithelial barrier function; v) immune system balancing and regulation. |
| **The human mammal is the target of environmental health risk assessments (e.g., toxicological safety).** | The human superorganism is the most relevant target for assessment/evaluation/safety determinations. Because microbes occupy the routes of exposure, if the microbiome is not included in environmental health risk determinations, the likely internal dose and potential for clinical pathology and disease is not actually known. |
| **Noncommunicable diseases (NCDs) are completely noncommunicable.** | Specific microbe transfer appears to promote certain NCDs. Such transfer is more likely when recipient microbiomes have been degraded (e.g., reduced colonization resistance). |

# Graphical Abstract: Enhancing Human Superorganism Ecosystem Resilience by Holistically 'Managing Our Microbes'

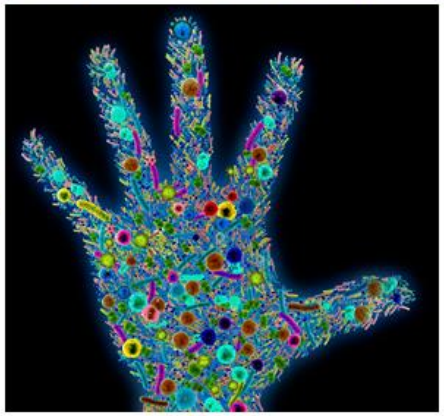
(Coleman et al., 2021. *Applied Microbiology)*

## Building Resilient Gut Ecosystems to 'Manage our Microbes'

### Healthy Human Superorganism

**Defining Exposures**

- Diet ↑ in whole fresh foods with beneficial microbes, prebiotic fibers
- RDAs for vitamins and microbes?



**Expected Outcomes**

- Healthy gut microbiome;
  ↑ colonization resistance;
  ↓ pathogen blooms;
  ↑ immune defenses to clear pathogens;
  ↓ health risks
- Well-primed immune system balances inflammatory responses to microbes in environment

### Dysbiotic Human Superorganism

**Defining Exposures**

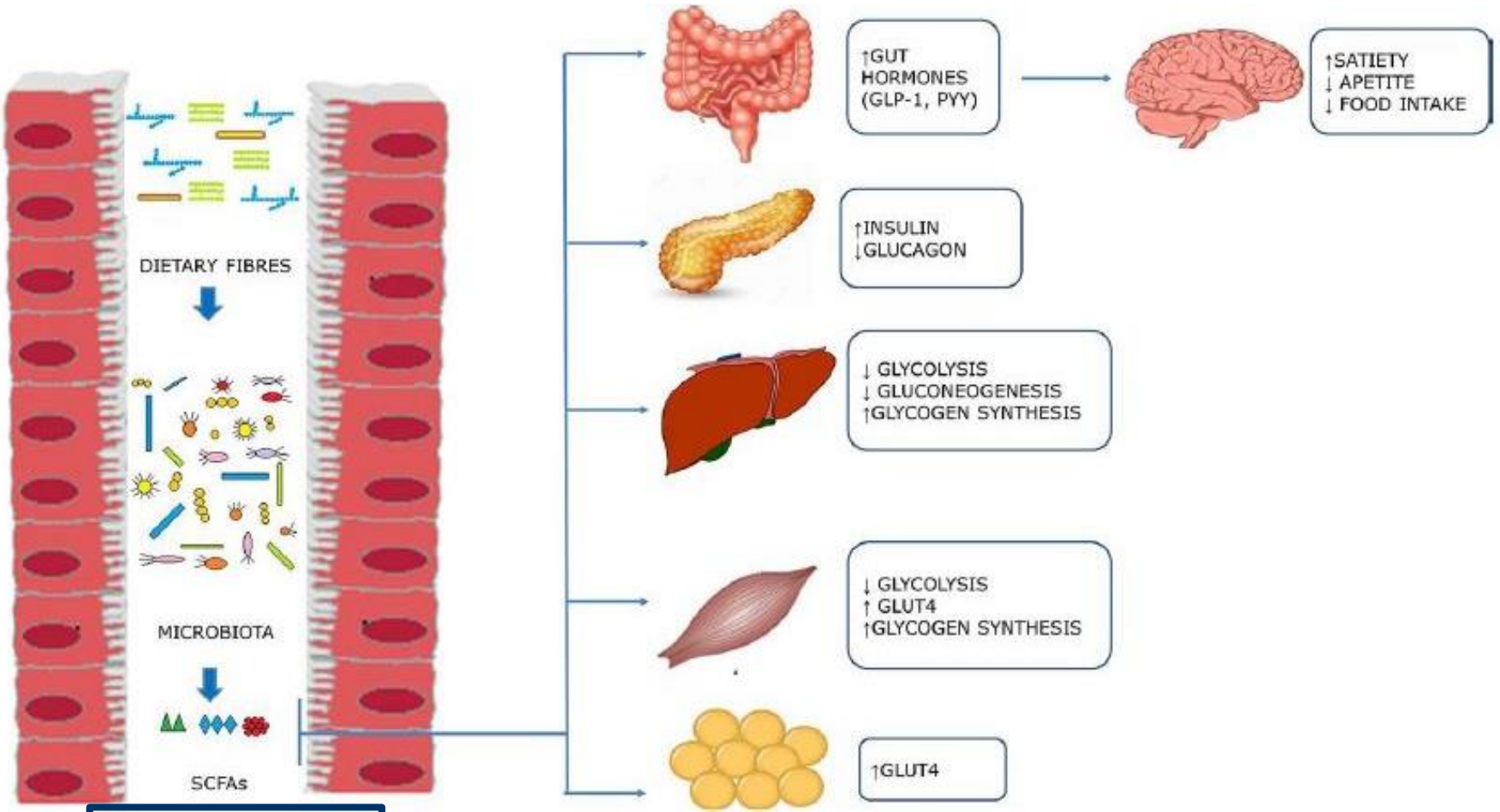
- Diet ↑ in processed foods, ↓ in beneficial microbes, prebiotic fibers
- Pharmaceuticals
- Open niches for opportunistic pathogens

**Expected Outcomes**

- Dysbiotic gut microbiome;
  ↓ colonization resistance;
  ↓ capability to assist immune defenses to limit, clear pathogens;
  ↑ pathogen blooms; ↑ health risks
- Imbalanced immune system;
  ↑ inflammatory responses;
  ↑ susceptibility to pathogens;
  ↑ damage host

# Diet Affects Whole Superorganism:

## Understanding Gut-Lung-Brain Axis




**Short Chain Fatty Acids** (e.g., butyrate, propionate)

**Salamone** et al., **2021**. The relationship between gut microbiota, short-chain fatty acids and **type 2 diabetes** mellitus: the possible role of dietary fibre. *Ageing Research Reviews*

# Cross-Talk Between Gut Microbiota, Immune System, Lungs

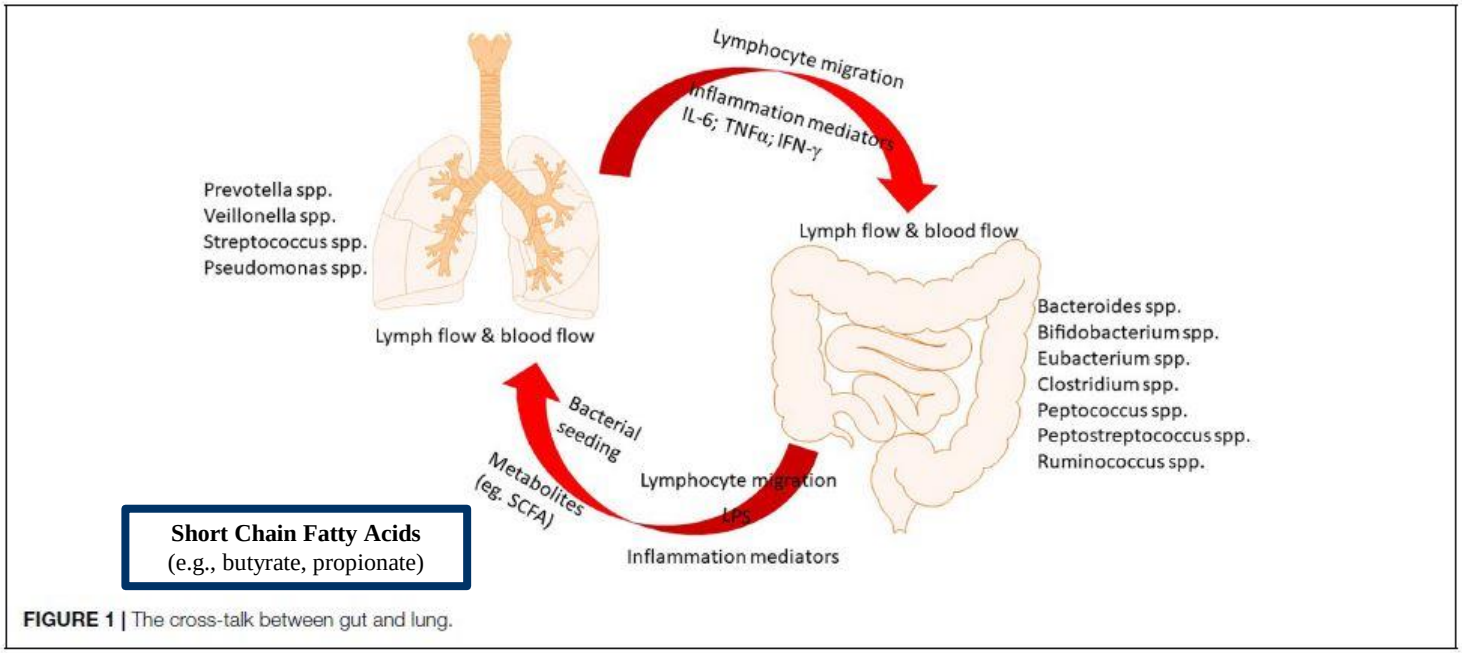



**FIGURE 1 |** The cross-talk between gut and lung.

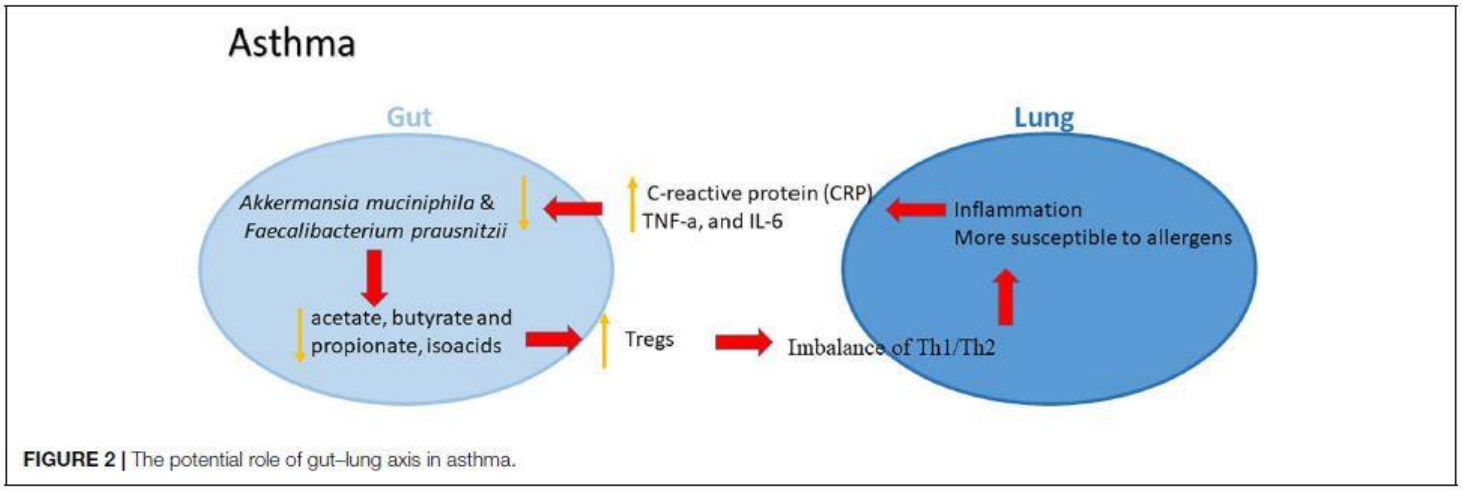



**FIGURE 2 |** The potential role of gut–lung axis in asthma.

**Zhang** et al., **2020**. The Cross-Talk Between Gut Microbiota and Lungs in Common Lung Diseases. *Cell Reports*

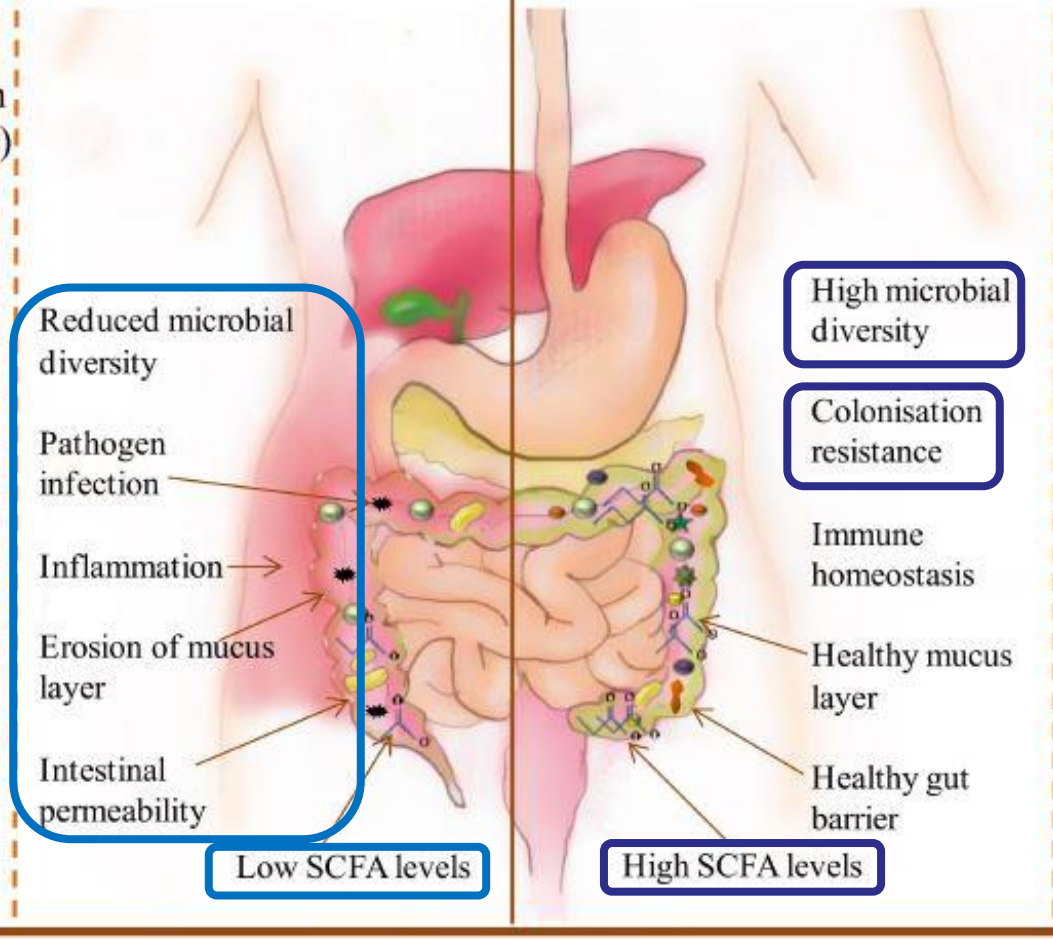
# ‘Managing our Microbes’ for Health and Resilience

**Poor Quality Diet**

Predominant foods

- Animal-derived protein (meat & processed meat)
- Saturated fats
- Refined grains
- Sugar
- Salt
- Alcohol
- Corn-derived fructose

**Consequences**




**Healthy Diet**

Predominant foods

- Fruits
- Vegetables
- Fibre
- Plant-derived protein
- MUFAs
- n-3 PUFAs
- **Whole dairy**
- **Animal/fish fats & proteins**

**Mills** et al., **2019**. Precision Nutrition and the Microbiome, Part I: Current State of the Science. *Nutrients*

# Resilience of Healthy Gut Ecosystem

- Ability of the system/ecosystem to sustain or restore its basic functionality following a perturbation, challenge, or stressor

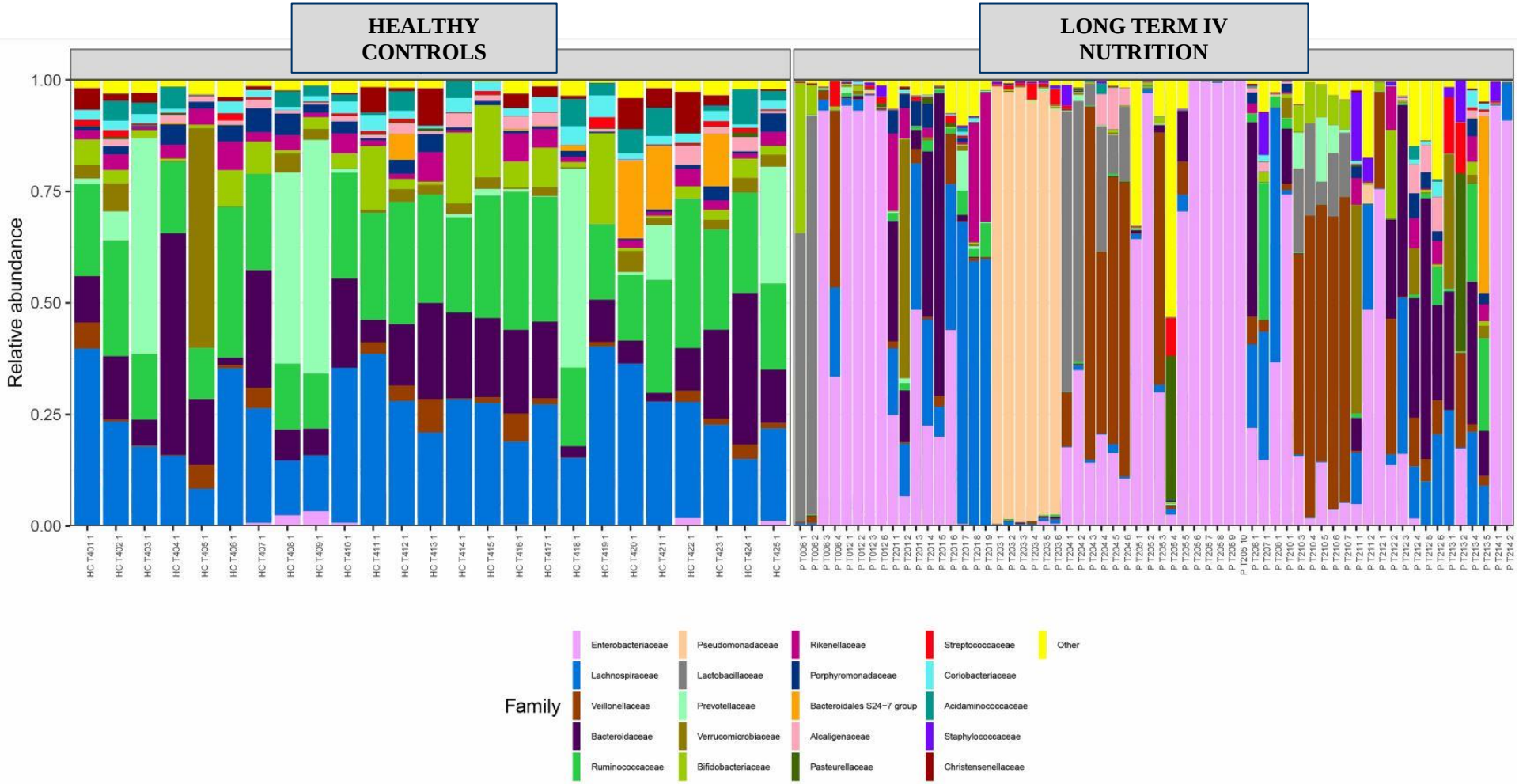
- Healthy **gut ecosystem** particularly complex, **high diversity and richness** (over 2,000 species representing 12 phyla; 9 dominant genera) and **functional redundancy**, stable to small perturbations (Perez-Cobas et al., 2013; Anwar et al., 2021)

- Stressors for gut resilience include pharmaceuticals, malnutrition, and diets low in fiber and enriched in processed foods

**Pharmaceutical stressors: antibiotics, laxatives, NSAIDs, proton pump inhibitors (PPIs), AND polypharmacy**

# Diverse Gut Microbiota in Healthy Children, Blooms in Pediatric Disease

(profound dysbiosis)




**Neelis** et al., **2021**. Gut microbiota and its diet-related activity in children with IF [intestinal failure] on long-term parenteral nutrition [intraveneous]. *Journal Parenteral Enteral Nutrition*

# Superorganisms Differ in Susceptibility, Resistance, Severity

- **Typical 20th century assumptions:** neonates, infants, young children highly susceptible to all infectious diseases

- **UNTRUE** for ***C. difficile*** **infection (CDI):**
  - consistent body of evidence from nearly 10 decades of observations of **high rates of asymptomatic colonization**, **very rare symptomatic CDI** in neonates and infants (Smith et al., 2020) versus
  - **disproportionately severe affects for elderly** in nursing home environments (Haran et al., 2021)
  - rising incidence, severity (~500,000 annual US cases, 1/5 severe with recurrence)

- **TRUE** for ***Staphylococcus aureus*** in **hospitalized** neonates, infants**:**
  - early life dysbiosis in nasal, gut, lung microbiota set up immune system for inflammation in the lung and chronic conditions including asthma (Khamash et al., 2018)

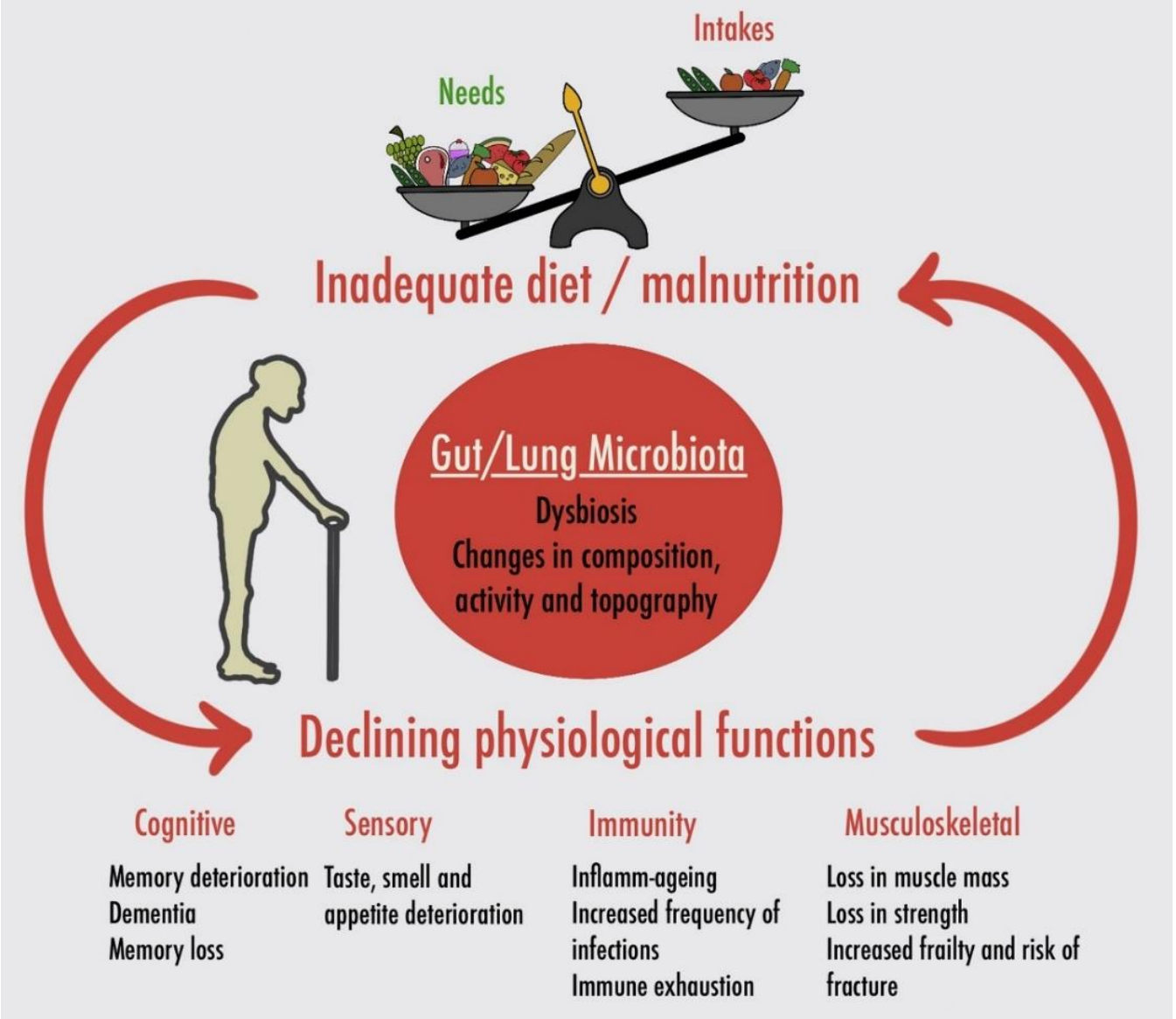
**Consider Pathogen-Microbiota-Host-Environment-Specific Dose-Response Models**

# Primary Risk Factors for Hospital Acquired Diarrhea (HAD), *C. difficile* Infection (CDI)

1. Exposure to **antibiotics**, particularly broad spectrum**, polypharmacy** (i.e., antibiotic + proton pump inhibitors), **chemotherapy**
2. Duration **hospitalization** (risk increases each day)
3. Advanced **age**
4. Underlying **co-morbidities**
5. Vitamin D deficiency
6. GI tract manipulation (i.e., GI tube insertion, GI surgery)

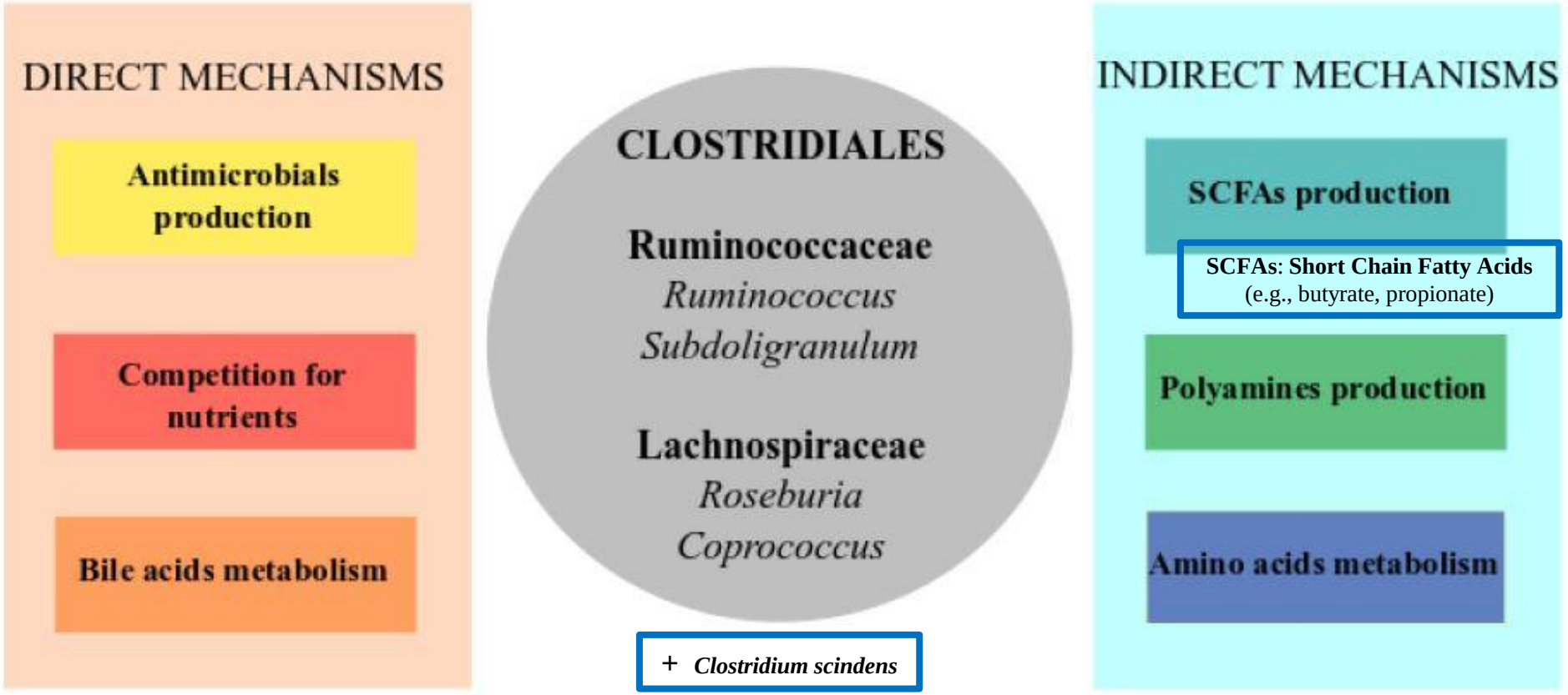
**Durham** et al., **2020**. Navigating changes in Clostridioides difficile prevention and treatment *Journal of Managed Care + Specialty Pharmacy*

# Interconnections of Diet and Dysbiosis Contribute to Physiological Declines with Aging



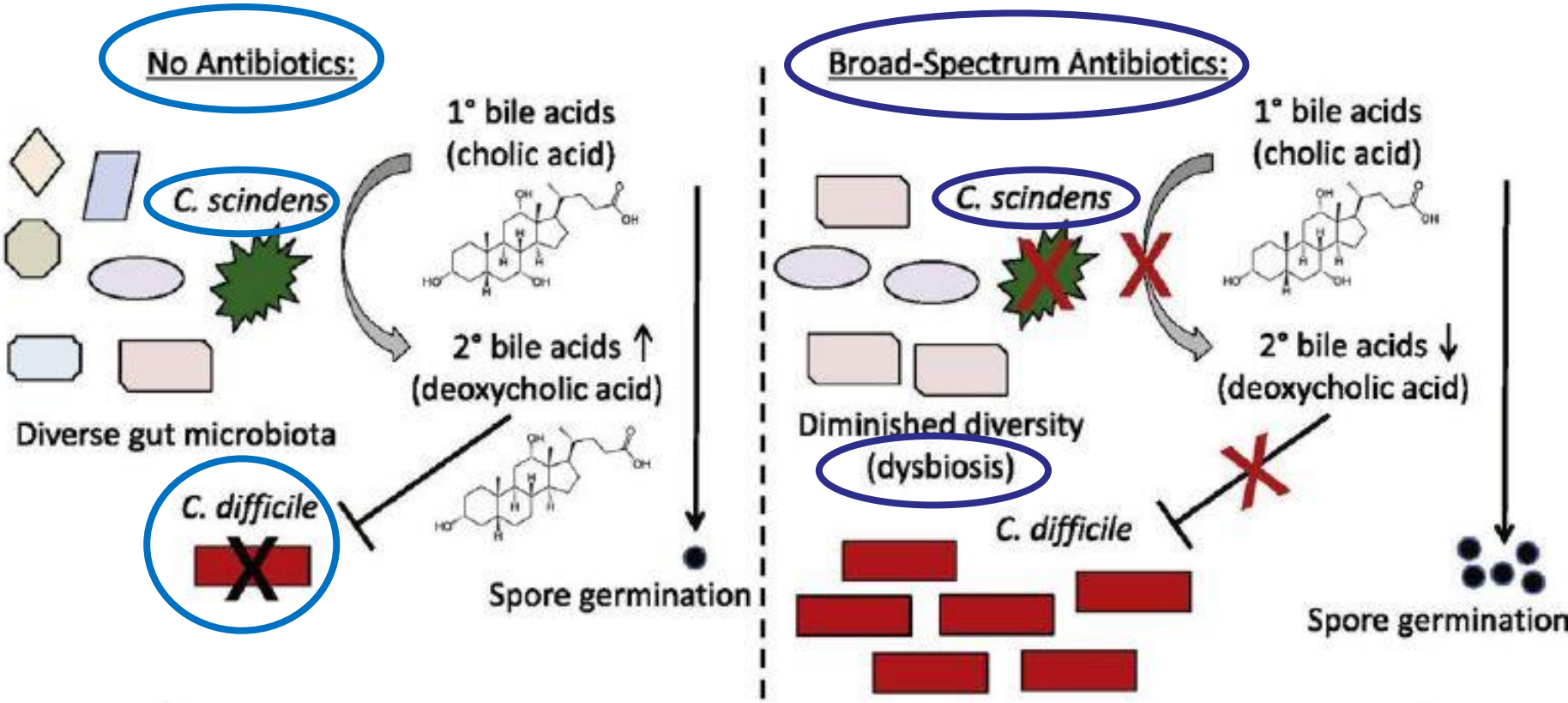

**Saint-Criq** et al., **2021**. Dysbiosis, malnutrition and enhanced gut-lung axis contribute to age-related respiratory diseases. *Ageing Research Reviews*

# Colonization Resistance to CDI: Related Commensals




**Perez-Cobas** et al., **2015**. Colonization Resistance of the Gut Microbiota against Clostridium difficile. *Antibiotics*

# More on Related Commensals




**Translational implications:**

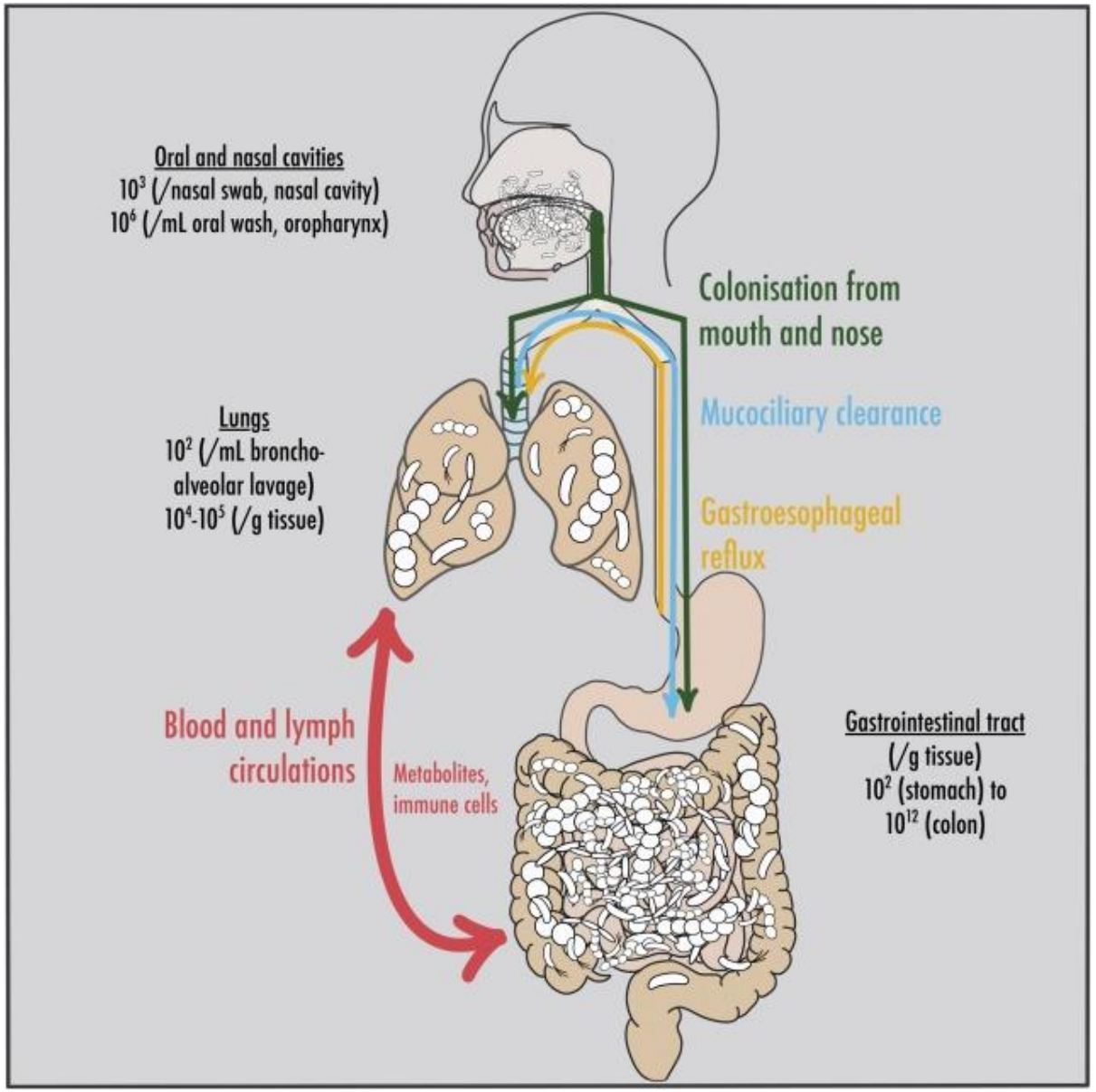
Spare *C. scindens via* narrow-spectrum antibiotics

and/or

Restore *C. scindens via* synthetic fecal microbitoa transplantation (probiotics)

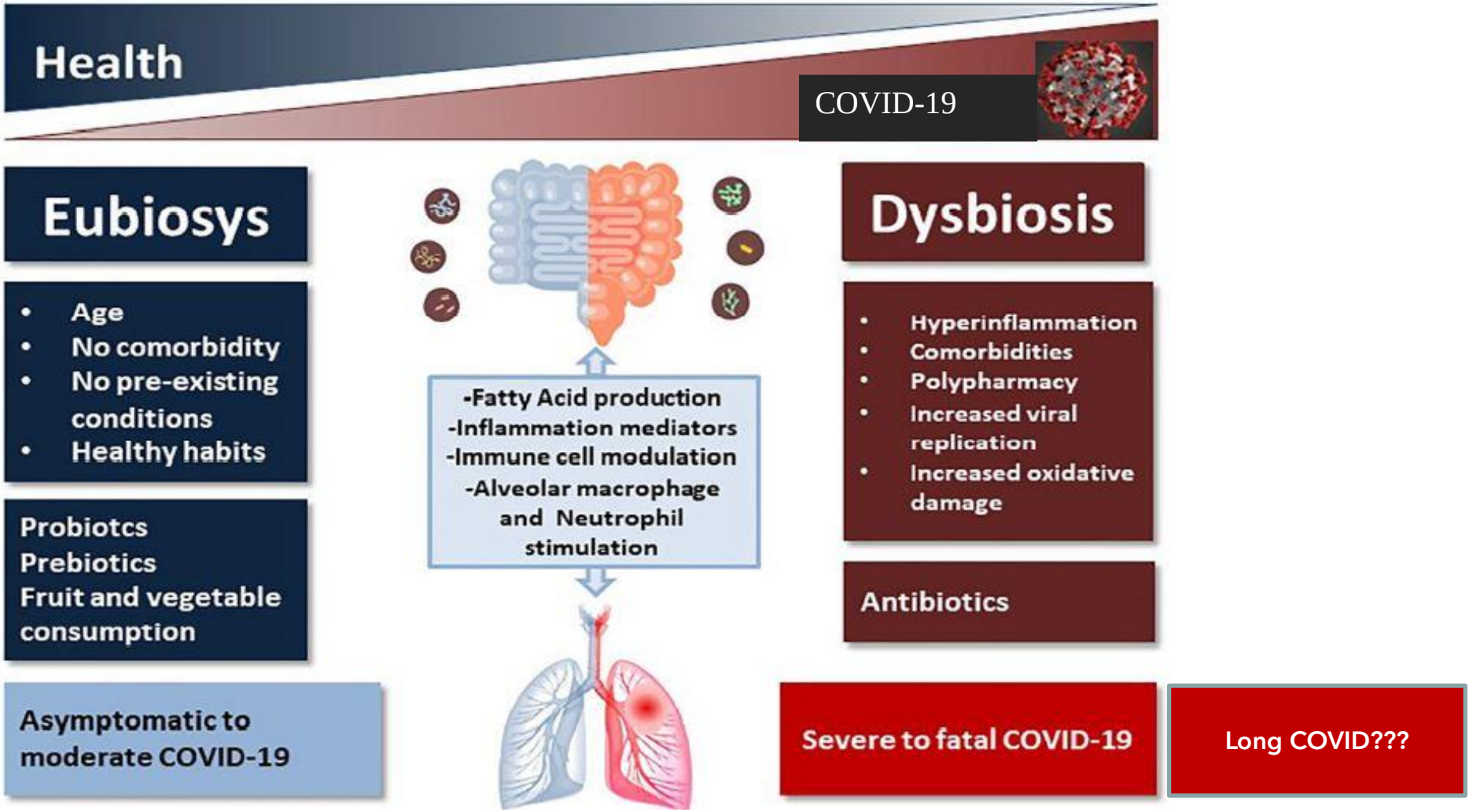
**Greathouse**, **2015**. Dysfunctional Families: Clostridium scindens and Secondary Bile Acids Inhibit the Growth of Clostridium difficile. *Cell Metabolism*

# Understanding Gut-Lung Axis



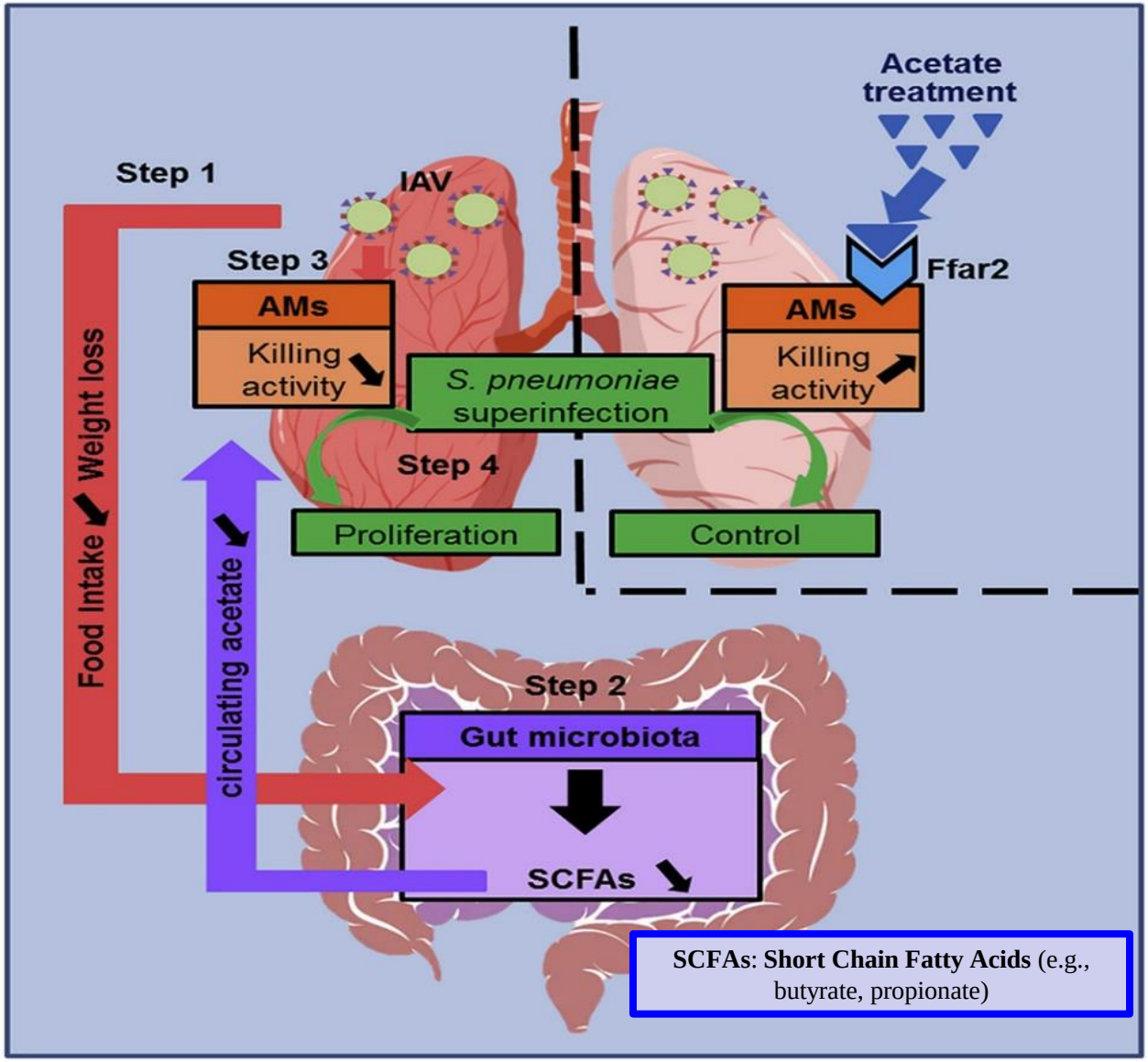

**Saint-Criq** et al., **2021**. Dysbiosis, malnutrition and enhanced gut-lung axis contribute to age-related respiratory diseases. *Ageing Research Reviews*

# Building on Gut/Lung Microbiota Studies




**Zeppa** et al., **2020**. Gut Microbiota Status in COVID-19: An Unrecognized Player?
*Frontiers in Cellular and Infection Microbiology*

# Gut Dysbiosis, Influenza, Pneumonia




**Sencio** et al., **2020**. Gut dysbiosis during influenza contributes to pulmonary pneumococcal superinfection through altered SCFA production. *Cell Reports*

# Section 2 Summary

1. The **natural microbiota** of foods (e.g., breast milk, bovine milk) **competes** with pathogens, **suppresses** or **eliminates** pathogen growth, and reduces pathogen survival under certain conditions

2. Gut microbiota **competes** with pathogens for **space** to **adhere** in the gut and for **resources** limiting growth important in simulating exposure assessment

3. Advancing knowledge of the gut microbiota **challenges** common **simplifying assumptions** for **modeling pathogen dose-response relationships**

# Section 2 Summary

4. The gut microbiota of **healthy superorganisms** trains and maintains balanced immune systems and provides **colonization resistance**, innate protection against pathogens under normal conditions.

**5.** **Antibiotic** administration disrupts the healthy microbiome, causing **dysbiosis** that **increases susceptibility** to many pathogens and left-shifts dose-response curves.

6. Microbiome studies building on **traditional microbial ecology** (Lotka-Volterra equations) reveal groups of microbes in humans and mice associated with **resistance** and **susceptibility** to *Clostridium difficile* and other pathogens.

# SECTION 3: INCORPORATING MICROBIOTA INTO 21ST CENTURY RISK ANALYSIS

# Common Risk Management Worldview in 1990s:

## Eliminate Bacteria in Foods at Genus or Species Level




## Current decade: shifts in food safety management and education

- **USDA FSIS**
  - monitors by **Whole Genome Sequencing** since **2015**; high variability in virulence profiles for foodborne pathogen isolates
  - Collaborating since **2022** with genomics experts on including virulence gene sequence comparisons in hazard, dose-response, and risk assessment, moving away from recalls or diversions (**high food waste** for many low risk foods) based on **presence of Genus *Salmonella***

- **USDA, FDA, Partnership for Food Safety Education** promote use of **thermometers** in **refrigerators** to maintain food temperatures **unlikely to permit pathogen growth** (4.4˚ C/ 40˚ F )

- **EFSA (2015).** Scientific Opinion on the Public Health Risks Related to the Consumption of Raw Drinking Milk.

# *Salmonella* Prevalence Alone Is Not a Good Indicator of Poultry Food Safety

(Oscar, 2021, *Risk Analysis 41*(1))

## USDA/FSIS regulates raw poultry meat based on the genus *Salmonella* alone, a variable insufficient to predict safety

- **Risk of salmonellosis** was **significantly** ($p > 0.05$) affected by:
  - **Prevalence**
  - **Number** in 26 samples collected in 2018
    - *Salmonella* not detectable to 40 bacteria in 25 g ground turkey samples
    - Natural microbiota 25,000 to 250,000,000 bacteria in same samples
  - **Virulence**
  - Incidence and extent of **undercooking**
  - Food **consumption behavior**
  - **Host resistance**

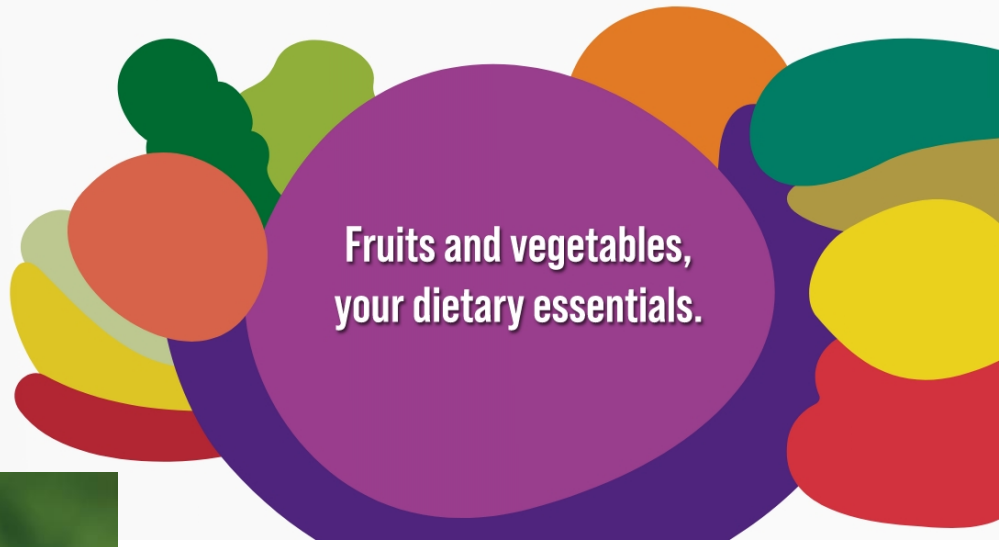
  but was **not affected** by **serving size**, **serving size distribution**, **or total bacterial load** of ground turkey when all other risk factors held constant
- Prevalence **not correlated** ($r = -0.39$; $p = 0.21$) with salmonellosis risk (other factors not held constant)
- Need for more holistic approach for modeling complex food systems, developing alternative risk management strategies and scenarios, and monitoring relevant predictors of safety

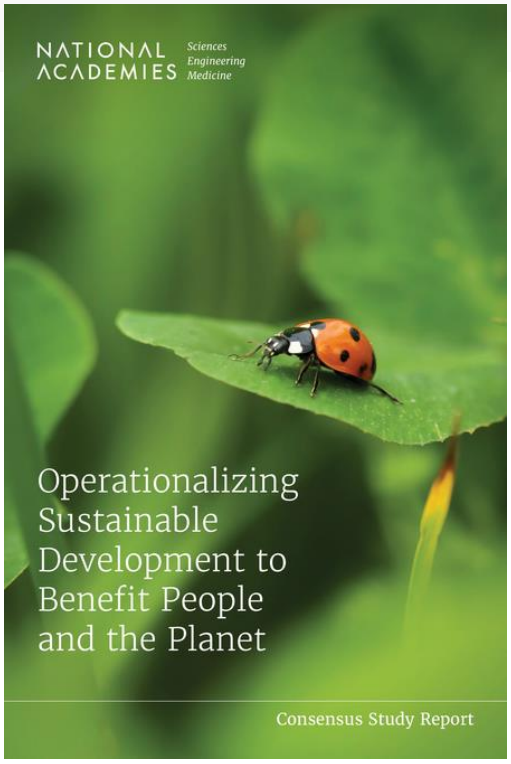
# Sustainability, Justice, Regenerative Agriculture

Food and Agriculture Organization of the United Nations

SUSTAINABLE DEVELOPMENT GOALS

INTERNATIONAL YEAR OF
FRUITS AND VEGETABLES
2021




- Food security affects 2.4 billion people worldwide and 1 in 10 people in the US
- Current US industrialized food systems generate 1/3 global greenhouse gas emissions and 70% global water use
- Need for holistic reforms for food systems (local regenerative agriculture) to balance:
  - Food safety and food security
  - Costs, benefits and risks for human and environmental health, cultural, social, economic and factors

# Combining Quantitative Risk Assessment of Human Health, Food Waste, and Energy Consumption: Next Step in the Development of the Food Cold Chain?

(Duret et al., 2019, *Risk Analysis* 39(4):906-925)

- Links prediction of product temperature in refrigeration processes, energy consumption, and predictive microbiology
- A cost-benefit analysis approach (DALYs) and 2 multi-criteria decision analysis methods (Analytic Hierarchy Process and ELECTRE III) used to rank 8 interventions related to human and environmental health, sustainability, and economics
- Utility high where no single '*a priori* optimal' solutions exists AND decision makers must prioritize among diverse criteria to identify 'best compromise'
- Setting refrigerator thermostat at 4° C best compromise between three potentially conflicting objectives
  - Food safety (risk of illness; estimated $50 billion US)
  - Food waste (spoilage, recalls for low-risk foods; estimated $218 billion US)
  - Economic loss (energy for refrigeration, recalls for low-risk foods)

# Policies for Listeriosis Reflecting Thresholds


Some governments regulate Ready-to-Eat Foods that:

**support growth** as unsafe **(adulterated)** if **1 bacteria or colony forming unit** (CFU)/mL *Listeria* is detected.

**do not support growth** as **adulterated** only if **≥100 CFU**/mL *Listeria* is detected.

- No evidence for growth**/no growth** of pathogens in raw milk at normal levels (typically 1 to 10 CFU/mL)
- If no growth for *Listeria,* **raw milks <100 CFU/mL** could be considered **unadulterated** (acceptable or tolerable or 'safe')

# Alternative Approaches to the Risk Management of *Listeria monocytogenes* in Low Risk Foods

(Farber et al., 2021, *Food Control* 123:107601)

Alternative perspectives on microbiological criteria for foodborne listeriosis

- US FDA: 'zero tolerance' (ZT) for Lm in RTE foods (declared adulterated based solely on pathogen presence using 2-class sampling plan)
- Canada, EU: 100 cfu/g for low risk foods suppressing growth of Lm using more flexible 3-class sampling plan

Multiple studies on thresholds for innate resistance to listeriosis (Buchanan et al., 2017; Rahman et al., 2016, 2018, 2020)

**Key findings:**

- **FDA** (blanket ZT) vs **FSIS** (alternative approaches: Gallagher et al, 2003; 9 CFR Part 430, 68FR34208-34254)
- ZT **very strong disincentive** for industry testing contact surfaces, finished product
- **benefits not recalling low-risk foods** not supporting Lm growth and containing low levels include:
  i) not wasting limited industry and regulator resources;
  ii) not losing consumer confidence,
  iii) maintaining a secure and sufficient food supply,
  iv) decreased food waste,
  v) avoiding negative effects on the environment, and
  vi) avoiding unnecessary costly food recalls
- **Recommendations**
  i) use alternate sampling approaches for low-risk foods;
  ii) use big data to better inform microbial risk assessments;
  iii) perform risk-benefit assessments; and
  iv) develop novel consumer food handling/risk communication strategies

**The application of 'zero tolerance' for Lm appears to reflect ideology, not science.**

# Policy Responses to Foodborne Disease Outbreaks in the US (and Germany)

(Meagher, 2022. Agriculture and Human Values 39:233–248
https://doi.org/10.1007/s10460-021-10243-9)

**Social construction of pathogenic *E. coli* outbreaks, ABSENT public engagement, deliberation**

**FDA: 2006 leafy greens outbreak** (276 illnesses, 5 deaths)

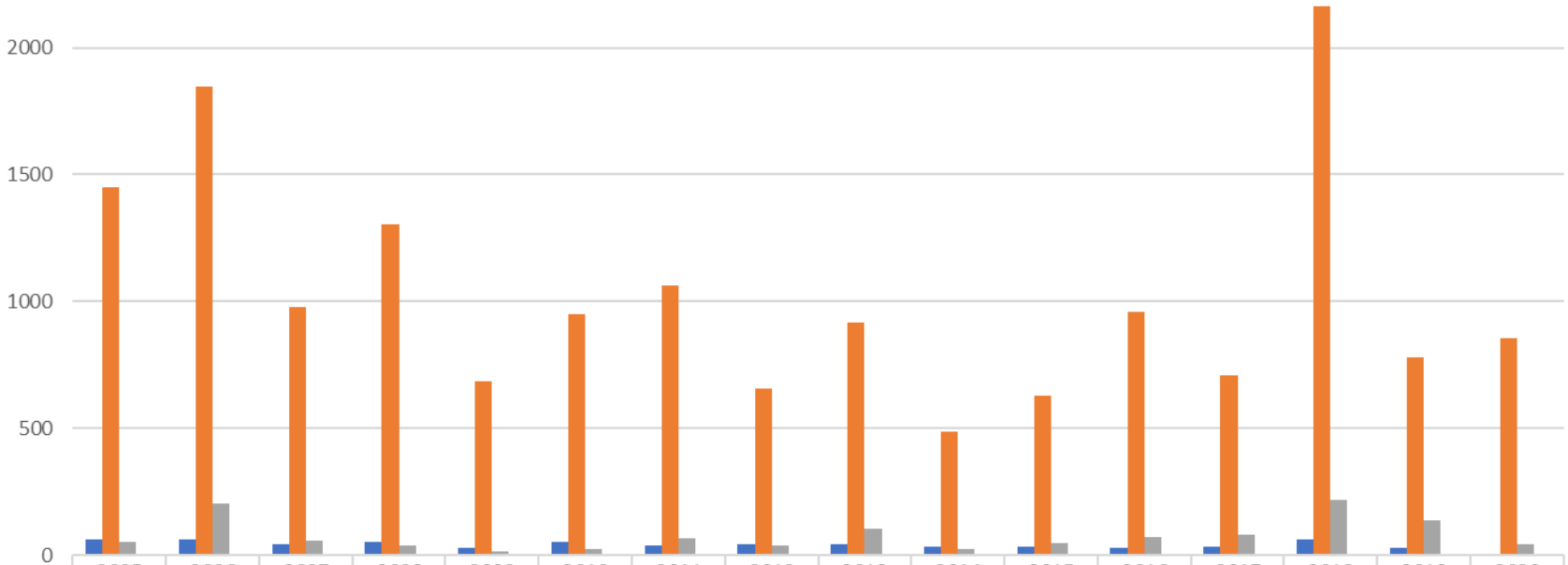
- Disregarded evidence of failures at processing facility, framed outbreak as an **agricultural problem**
- Warned consumers to **avoid eating fresh spinach from any source** even though contaminated product quickly traced to **CA grower**
- Targeted **farm-level food safety program**, blaming farmers or 'nature', pursuing **technical fixes on farms** rather than **holistic structural reforms** of both **production and processing industries**
- Unanticipated consequence of actions: interfered with **farm conservation practices**, interventions **too costly for small producers**, furthering **Big Ag 'regime'**, continuing leafy green outbreaks, illnesses, hospitalizations, and deaths (CDC, 2021)

**Table 1** Five alternative policy options

| Policy options | Example actions |
|---|---|
| 1. Consolidate food safety authority | Centralize authority over standard-setting and enforcement in a single agency |
| 2. Abandon risky food products | Substitute less-risky products for bagged greens and raw sprouts |
| 3. Reduce scale or speed of production | Hand-harvest leafy greens; decentralize processing; lower sprouting temperatures |
| 4. Regulate cattle | Treat manure; increase distance between feedlots and produce fields; cull "super-shedder" cattle |
| 5. Do nothing | N/A |

# CDC Data on Leafy Green Outbreaks

(CDC, 2021)




| | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Outbreaks | 62 | 64 | 45 | 53 | 30 | 54 | 41 | 45 | 44 | 36 | 34 | 32 | 33 | 61 | 30 | 7 |
| Illnesses | 1,450 | 1,848 | 979 | 1,302 | 684 | 949 | 1,064 | 657 | 917 | 486 | 628 | 961 | 709 | 2,165 | 781 | 854 |
| Hospitalizations | 55 | 204 | 57 | 40 | 18 | 27 | 68 | 40 | 106 | 25 | 51 | 73 | 82 | 219 | 139 | 46 |
| Deaths | 0 | 5 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 4 | 1 | 0 | 4 | 5 | 0 | 0 |

# Leveraging Risk Assessment for Foodborne Outbreak Investigations: Quantitative Risk Assessment-Epidemic Curve Prediction Model

(Mokhtari et al., 2022, *Risk Analysis* 1-15)

## FDA perspective

- **Objective**: assess possible **root causes** of foodborne outbreaks
- Simulate lettuce supply chain for whole and fresh cut lettuce
- Consider time-dependencies and scenarios representing post-harvest processing conditions and practices
- Comparison of simulated outbreak patterns with retrospective data from past outbreaks
- Predicted epidemic curves similar in size to past outbreaks, not strongly influenced by facility processing/sanitation conditions
- Could be used to explore potential root causes

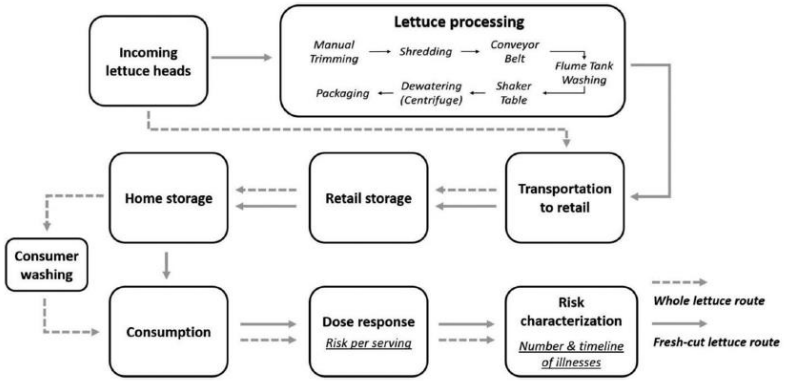



FIGURE 1 Flow diagram of the FDA leafy green quantitative risk assessment- epidemic curve model (FDA-LG QRA-EC). Solid arrows represent the supply chain for whole lettuce; Dashed arrows represent the supply chain for fresh-cut lettuce

## Questions

- Unclear if **multiple hurdles** to suppress pathogen survival and growth were examined
- No consideration of **leafy green microbiota**
- No alternatives to **conservative DR assumptions** based on sparse data
- **Uncertain** conclusions may reflect **correlative** or **causal** relationships

Dense diverse leafy green microbiota ($8x10^3$ to $6x10^8$ cfu/g)

*Pseudomonas, Chryseobacterium, Pantoea, Flavobacterium, Ralstonia, Stenotrophomonas, Erwinia, Xanthomonas, Serratia, Enterobacter, Bacillus, Staphylococcus, Acinetobacter, Alkanindiges, Comamonas, Limnobacter, Pelomonas*

(Mogren et al., 2018. The Hurdle Approach... *Frontiers in Microbiology 9:1-20)*

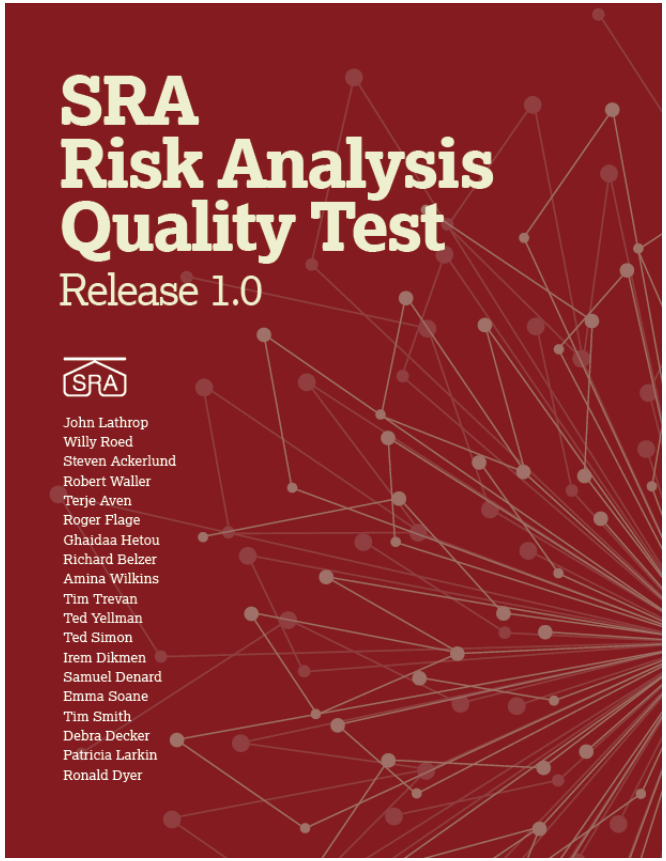
# Risk Analysis Quality Test (RAQT) of Society for Risk Analysis

Need for developing the RAQT identified by leaders of the ARM specialty group in **2015**

Subsequent round table panels and webinars involving many diverse risk practitioners of SRA

In **2021**, RAQT v 1.0 was released, with 19 risk practitioners listed on cover: available free at https://www.sra.org/resources/risk-analysis-quality-test/

Fifteen categories, including **76 specific yes/no questions**, highly relevant to quality analysis for chemical, microbial, and physical hazards



# Fifteen Categories of the RAQT

A. Framing the Analysis and Its interface With Decision Making

B. Capturing the Risk Generating Process (RGP)

C. Communication

D. Stakeholder Involvement

E. Assumptions and Scope Boundary Issues

F. Pro-Active Creation of Alternative Courses of Action

G. Basis of Knowledge

H. Data Limitations

I. Analysis Limitations

J. Uncertainty

K. Consideration of Alternative Analysis Approaches

L. Robustness and Resilience of Action Strategies

M. Model and Analysis Validation and Documentation

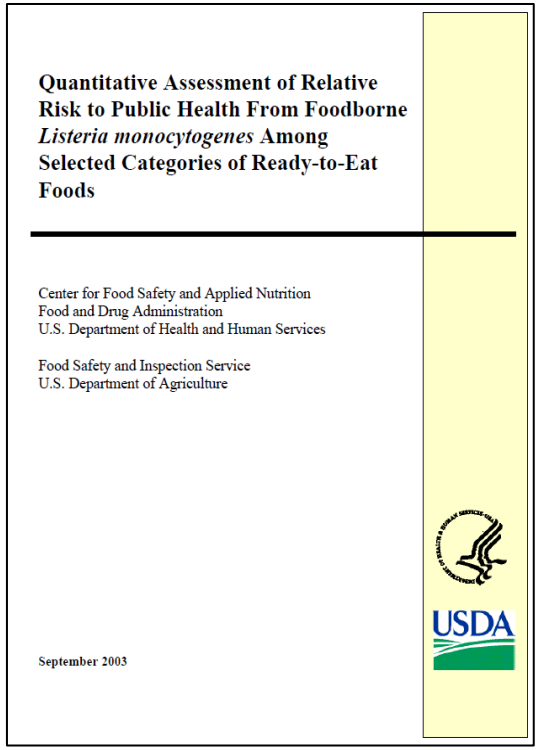
N. Reporting

O. Budget and Schedule Adequacy

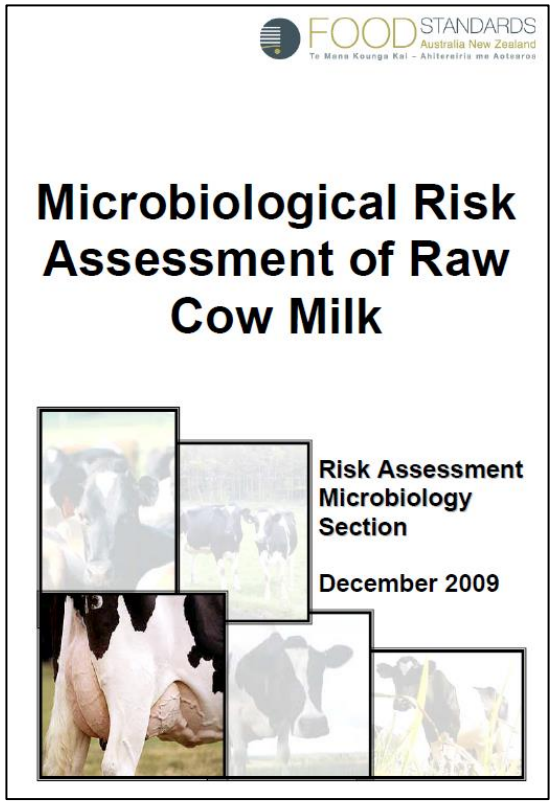
# Two QMRAs Evaluated using the RAQT

- **Joint FDA/FSIS, 2003**
  examine systematically available scientific data to estimate relative risk of **severe listeriosis** for US consumers of **23 RTE foods** (including **both raw** and **pasteurized milks**)

  (*Listeria monocytogenes* abbreviated **Lm**)




- **FSANZ, 2009**
  estimate risks and factors impacting risks along the production chain for **campylobacteriosis, listeriosis, pathogenic *E. coli*, salmonellosis** for Australian consumers of **raw milk**



# Findings from Applying the RAQT to QMRAs

1. **Both QMRAs failed all 15 categories, all 76 questions in the RAQT**
   - Evidence of bias, disconnection of QMRAs with risk management decision-making, risk communication, and stakeholder involvement on alternative risk management scenarios
   - Highest priority failure of both QMRAs: **Basis of Knowledge** (scientific evidence)
     - Failure to clearly communicate to decision makers where limitations of scientific knowledge (and its basis and strength) call for risk management strategies that take those limitations into account
   - Five categories with highest priority failures for raw milk assessments:

     G. Basis of Knowledge

     A. Framing the Analysis and Its Interface With Decision Making

     J. Uncertainty: Sources, Characterization, Implications for Risk Management

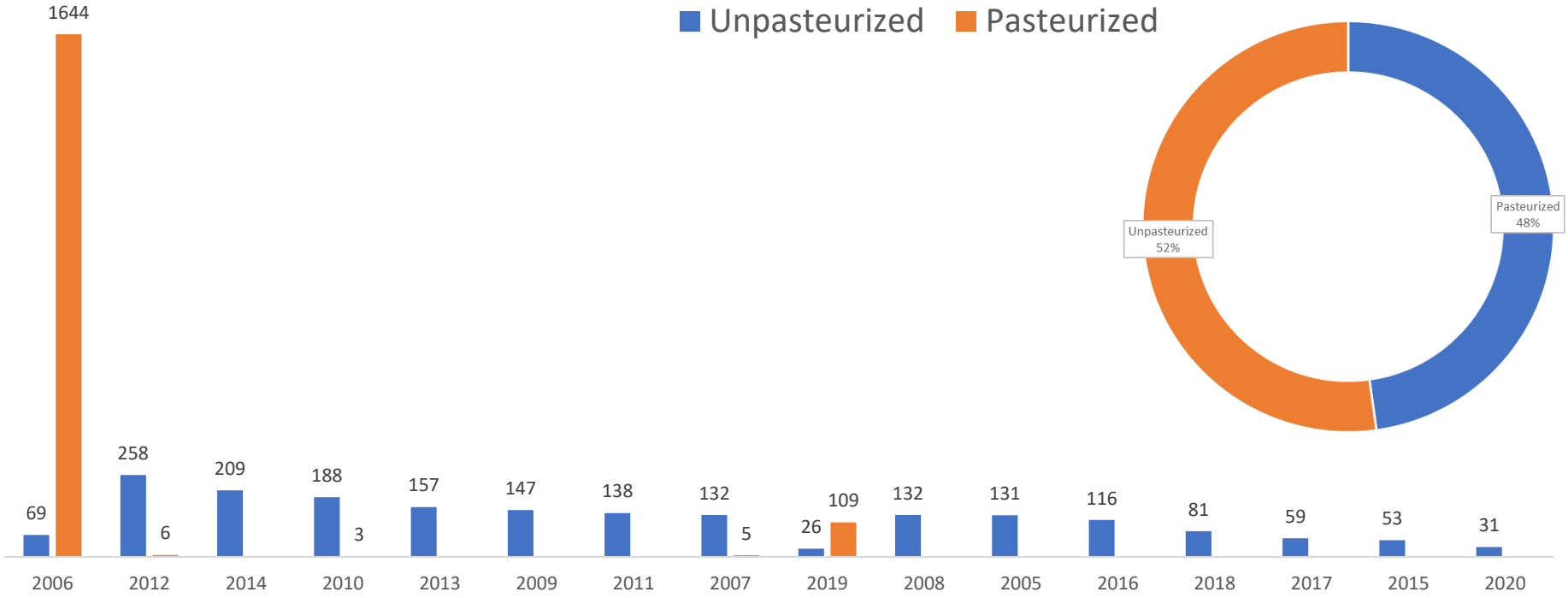
     D. Stakeholder Involvement

     C. Risk Communication

2. Some **scientific data** documented, some excluded or inappropriately pooled; policy **decisions** appear based in **ideology, politics**, NOT **scientific evidence**

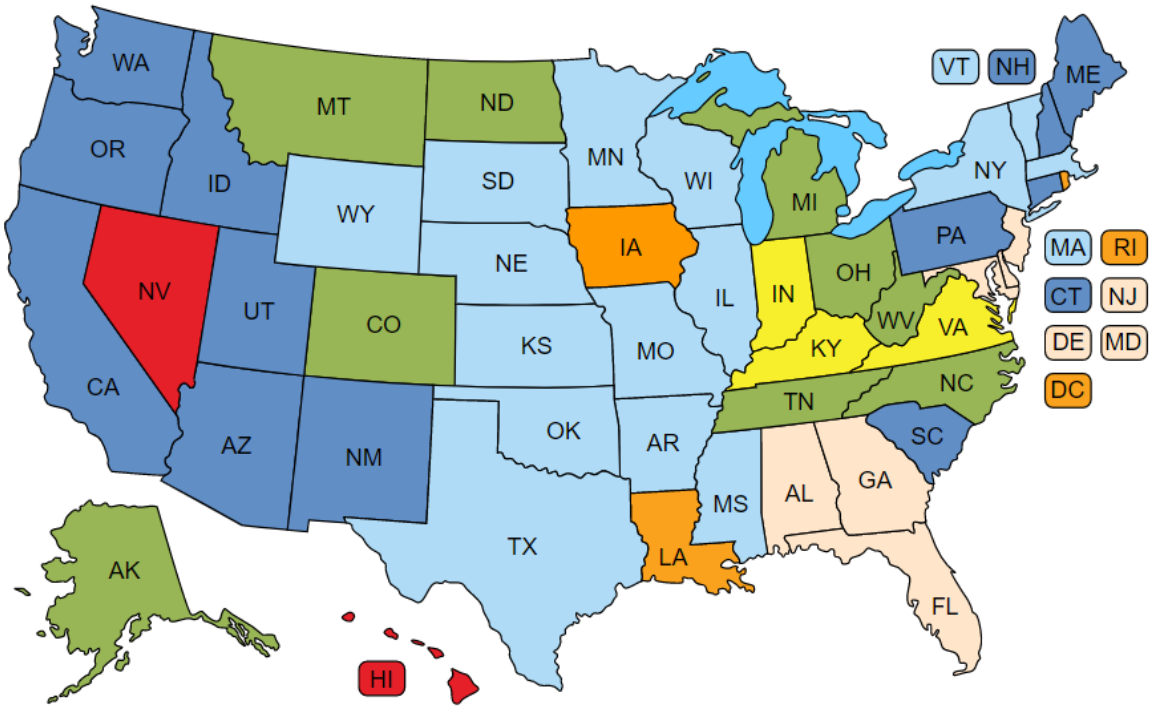
# Current Reality: US Epidemiologic Evidence Challenges Ideology, Zero-Risk Assumption for Pasteurized Milk

Illnesses associated with milk: **3,765 cases**, **48%** associated with **pasteurized** milk
(Source: CDC, 2021; 2005-2020)




Deaths Rare for Milk in N America: in 16 Years, 6 US Deaths (4 pasteurized, 2 raw), 4 Canadian Deaths (pasteurized)

# Real Milk Interactive Map on Legal Access to Raw Milks




**BLUE** Retail sales legal

**CYAN** On-farm sales legal — This category includes sales through delivery, at farmers market, and at any other venues where direct raw milk producer-to-consumer transactions are allowed.

**GREEN** Herdshares legal — by statute, regulation, court decision, or written policy

**YELLOW** No law on herdshares — The state is aware herdshare operations exist but has taken no action to stop them.

**PEACH/MELON** Sales of raw pet milk legal—farmers are selling raw pet dairy products

**ORANGE** Sales of raw pet milk legal—distributed only by national raw pet dairy manufacturers but there are no farmers selling raw pet milk

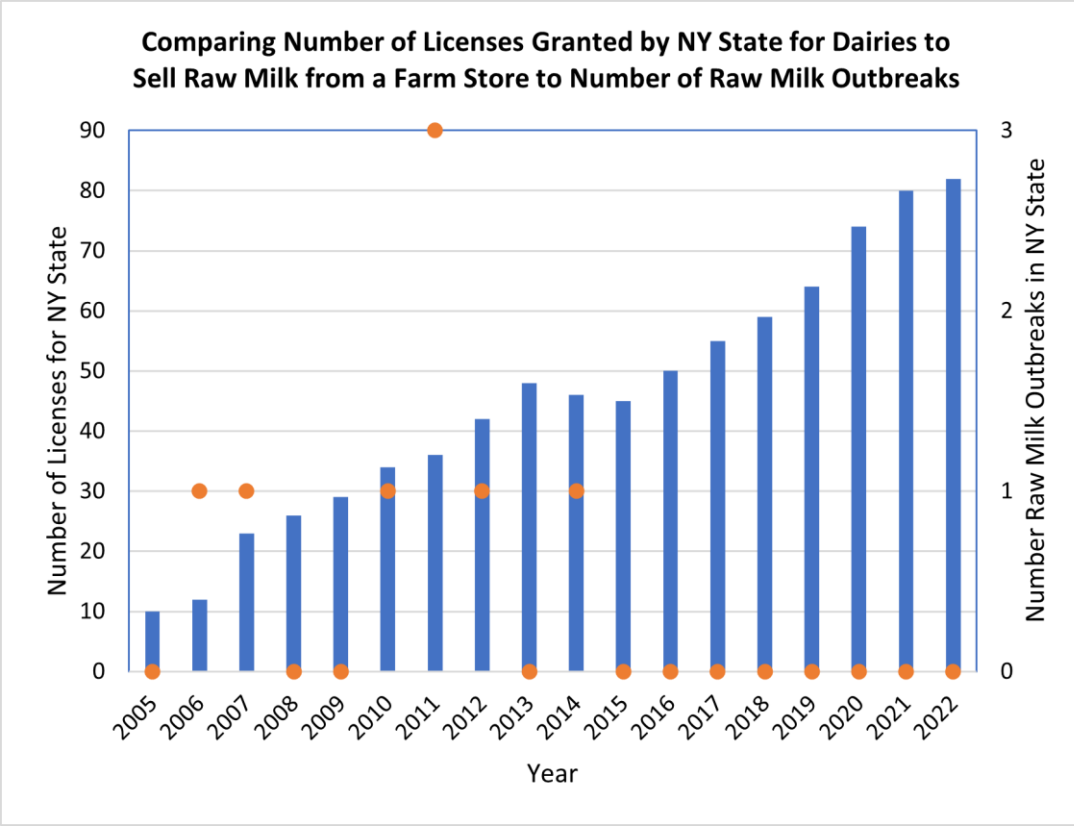
**RED** All sales and distribution of raw milk illegal — Nevada is the only state in this category; it allows the sale of raw pet milk but only if a toxic denaturant is added

https://www.realmilk.com/real-milk-legal-map/, last updated 11 Nov 2022

# Reality for 2022: NY State Licensed 82 Raw Milk Dairies

- NY State data on numbers of licenses for **on-farm** raw milk sales (obtained by FOIA)

- US CDC NORS outbreaks (2005-2020)
  - Eight NY state outbreaks in 16 years
    - 58 **campylobacteriosis** illnesses (4 hospitalizations, 0 deaths)
  - **No raw milk outbreaks** reported in NY state since **2014** despite increasing numbers of licenses for farms legally selling raw milk (data obtained by FOIA)
  - **Pathogens NOT associated with NY state outbreaks** (since at least 1998):
    - *Salmonella* spp.
    - Pathogenic *E. coli* (EHEC/STEC/VTEC)
    - *Listeria monocytogenes*



*applied microbiology*

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## Enhancing Human Superorganism Ecosystem Resilience by Holistically 'Managing Our Microbes'

Margaret E. Coleman; Rodney R. Dietert; D. Warner North; Michele M. Stephenson

*Appl. Microbiol.* **2021**, Volume 1, Issue 3, 471-497

*applied microbiology*

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## Examining Evidence of Benefits and Risks for Pasteurizing Donor Breastmilk

Margaret E. Coleman; D. Warner North; Rodney R. Dietert; Michele M. Stephenson

*Appl. Microbiol.* **2021**, Volume 1, Issue 3, 408-425

*applied microbiology*

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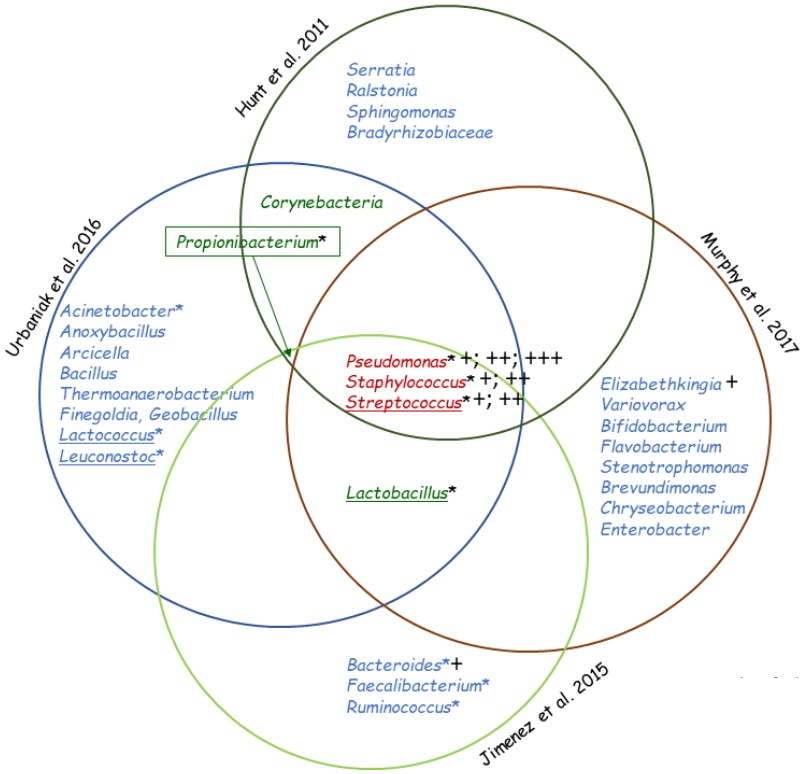
## Nourishing the Human Holobiont to Reduce the Risk of Non-Communicable Diseases: A Cow's Milk Evidence Map Example

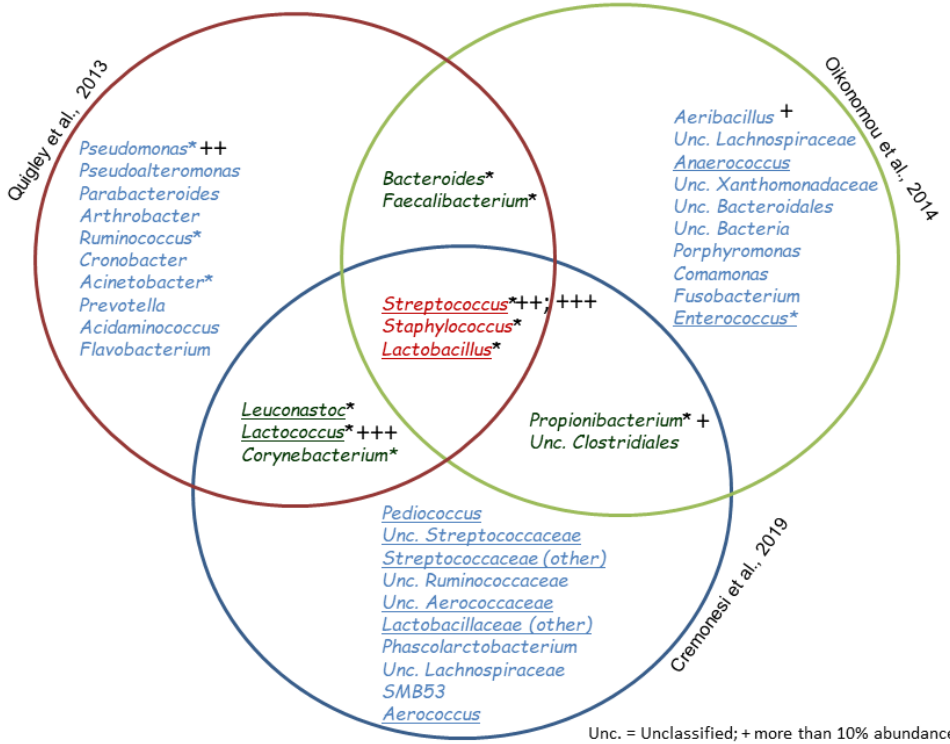
Rodney R. Dietert; Margaret E. Coleman; D. Warner North; Michele M. Stephenson

*Appl. Microbiol.* **2022**, Volume 2, Issue 1, 25-52

# Milk Microbiota Similarities

Dietert, Coleman et al., 2021c. Nourishing the Human Holobiont to Reduce the Risk of Non-Communicable Diseases: A Cow's Milk Evidence Map Example. *Applied Microbiology*




Unc. = Unclassified; + more than 10% abundance;
++ more than 25%; +++ more than 50%;
Underlined genera members of Lactic Acid Bacteria (LAB);
* Dominant genera in common for breastmilk and bovine milk

# Graphical Abstract: Examining Evidence of Benefits and Risks for Pasteurizing Donor Breastmilk

(Coleman et al., 2021a. *Applied Microbiology*)

## Benefits and Risks of Raw and Pasteurized Breastmilk

### Raw Breastmilk

photo by Kyle Nieber on Unsplash

↑ diversity of gut microbiota
↑ colonization resistance
↓ infectious and noninfectious diseases
↓ risk of childhood and maternal obesity
↑ developing nervous system
↑ cognitive development
↓ chronic disease

### Pasteurized Donor Milk

photo by Lucy Wolski on Unsplash

↓ diversity gut microbiota
↑ dysbiosis
↓ colonization resistance
↓ weight gain and growth
↑ risk of necrotizing enterocolitis
↑ risk of mortality
↑ risk of infectious and noninfectious diseases
↑ cost
↓ cognitive development
↑ chronic disease

# General View for Human Milk Bank Policies

- Rigorous donor screening methods similar to blood donation
- Some screen donor milk for other potential pathogens and indicators of contamination
- Some limits for pathogens/indicators (counts per mL) in donor milks (Omarsdottir et al., 2008)
  - <100,000 *Staphylococcus aureus*
  - <100 Enterobacteriaceae
  - 0 (below limit of detection) for potential pathogens

    *Listeria monocytogenes, Salmonella,* Group B/α-hemolytic *Streptococcus*, coagulase-negative *Staphylococcus*
- Most **pasteurize** donor milk (NOT Germany, Japan, Norway)

**Assumption:** ~~*Pasteurization*~~ *Minimizes Risks for NICU Infants*

# Benefits AND Risks for Vulnerable Population

## Human Milk Banks

provide **pasteurized** human donor milk to hospitalized preterm infants and sick/high risk infants

**Holder pasteurization** (heating to 62.5° C for 30 minutes) is required due to **perception**: possible presence of potential pathogens perceived as **'risky'**

**Yet Loss of Benefits for Pasteurized Milks in Clinical Studies around the World!**

- **Ford et al., 2019:** 74 preterm infants raw, 43 past donor (US, TX)
- **Sun et al., 2019:** 98 very preterm infants raw, 109 past donor (China)
- **Squires, 2017**: 302 low birth weight infants (US, WA)
- **Cossey** et al., **2013**: 303 very low birth weight infants (Belgium)
- **Strand** et al., **2012**: 335 infants and toddlers (Nepal)
- **Montjaux-Regis** et al., **2011**: 55 premature infants (France)
- **Schanler** et al., **2005**: 243 extremely low birth weight infants (US, TX)
- **Narayanan** et al., **1984**: 226 high risk, low birth weight infants (India)

# Evidence Map for Breastmilk Ecosystem

## Pro Supplemental Studies on Mechanisms

- **Vertical and Horizontal (Environmental) Transfer of Microbes** from maternal diet to gut, mammary tissues, milk, infant
  - Gregory 2016
  - Sawh 2016
  - Murphy 2017
  - Toscano 2017
  - de Andrés 2018
  - Ojo-Okunola 2018
  - Moossavi and Azad 2019
  - Van Deaele 2019
  - Wang 2020
- **Microbial Ecology**
  - Arroyo 2010
  - Fernandez 2016
  - Cacho 2017
- **Microbiome: Immune System Crosstalk**, indirect colonization resistance, recent reviews
  - Ward 2013
  - Chong 2018
  - Dietert 2018
  - van den Elsen, 2019

## Contra Supplemental Studies on Mechanisms

- **Pathogen susceptibility to innate defenses including microbiota**
  - Cacho and Lawrence 2017
  - Dietert 2018
  - Le Doare 2018
  - Ojo-Okunola 2018

supporting

- ***B-RA (Meltzer 2016), SR*** **(Miller 2018)**, ***SR/MA*** **(Villamor-Martinez 2018)**, ***CSs*** **(Sun 2019; Ford 2019)** demonstrated loss of benefits (protection against mortality, NEC, sepsis, other) for pre-term infants fed pasteurized donor milk or formula.

## Pro-Argument on Benefits of Raw Breastmilk

1. Extensive consistent evidence for dose-dependent protective effects compared to formula (or pasteurized donor milk) against incidence and severity of infectious diseases: ear and upper respiratory infections, diarrhea.
2. Extensive evidence for protective effects against non-communicable diseases: convincing for obesity; probable for asthma, celiac, Crohn's, diabetes, eczema, high blood pressure, ulcerative colitis, wheezing.

attenuating

- No studies identified that attribute benefits to specific raw milk microbes or microbial consortia.
- ***SR/MA*** of observational studies demonstrated pasteurized donor milk reduced bronchopulmonary dysplasia compared to formula; effect not observed in randomized trials (**Villamor-**Martínez **2018**).

supporting

- **CS (Bapistella 2019)** demonstrated lower CMV infection rates for mother's own breastmilk treated with short-term pasteurization than historical controls fed raw breastmilk.
- Policy paper on infectious diseases associated with mothers' & donors' breastmilk (**American Academy of Pediatrics, 2017**).

## Contra-Argument on Risks of Enteric Infections from Potential Pathogens in Raw Breastmilk

Limited evidence for normal breastmilk from healthy mothers causing infectious diseases in infants.

attenuating

- No **B-RA** or **QMRA, *SRs*, *MAs***, or ***CSs*** identified estimating risks of infectious disease transmission by breastmilk; review (**Gribble and Hausman, 2012**).
- ***CS*** **(Schanler, 2011)** demonstrated pathogen presence in breastmilk not predictive of illness in preterm infants.
- Long history of use of raw donor breastmilk in Norway (**Grøvslien and Grønn, 2009; Grøvslien 2020).**

## Evidence Basis

- 1 ***Benefit-Risk Assessment*** **(Meltzer 2013/16)**, 1 ***SR*** **(Miller 2018)**, 1 ***SR/MA*** **(Villamor-Martinez 2018)**, 2 ***CSs*** **(Sun 2019; Ford 2019)** citing extensive, consistent evidence
- 5 Other supporting or attenuating studies
- 18 Supplemental studies with evidence on plausible mechanisms

## Conclusions

1. Overall biological benefits associated with breastmilk clear, convincing, and conclusive, with supplemental studies on plausible mechanisms attributed to biologically active raw breastmilk
2. Evidence for assessing risks of pathogen infections in infants fed breastmilk from moms and donors limited and inconclusive

## Remaining Uncertainties

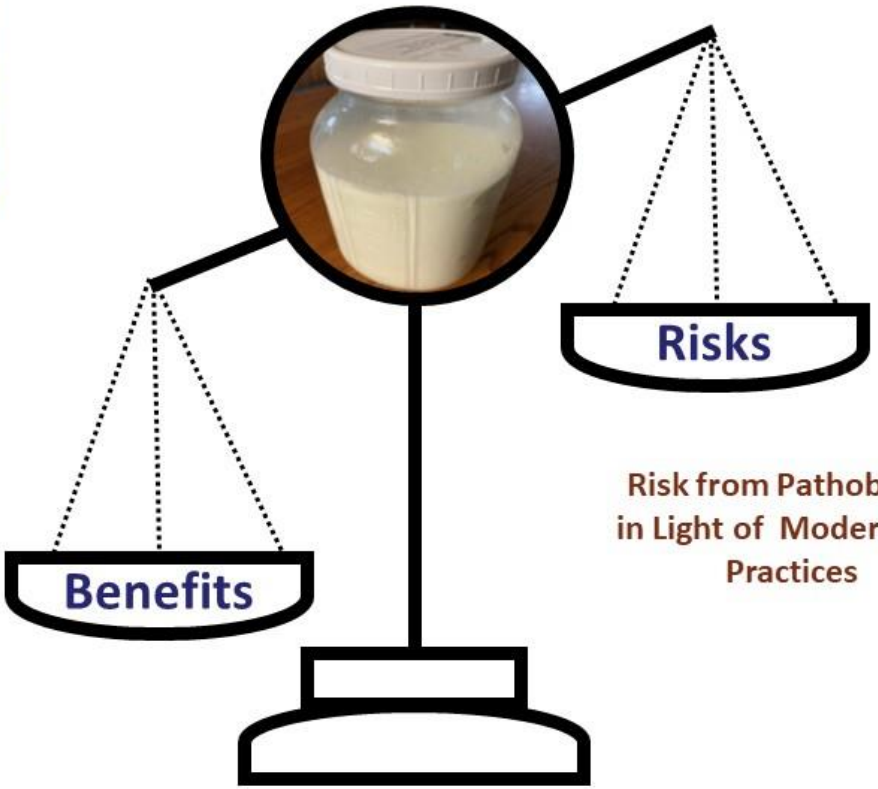
- How do milk microbiota function in protection against infectious and non-communicable diseases in infancy and later in life?
- Are presence or levels of potential pathogens in breastmilk predictive of illness in infants or mothers?
- Are there health benefits to pasteurizing donor milk for preterm or ill infants?

# Graphical Abstract: Nourishing the Human Holobiont to Reduce the Risk of Non-Communicable Diseases: A Cow's Milk Evidence Map Example

(Dietert et al., 2021)

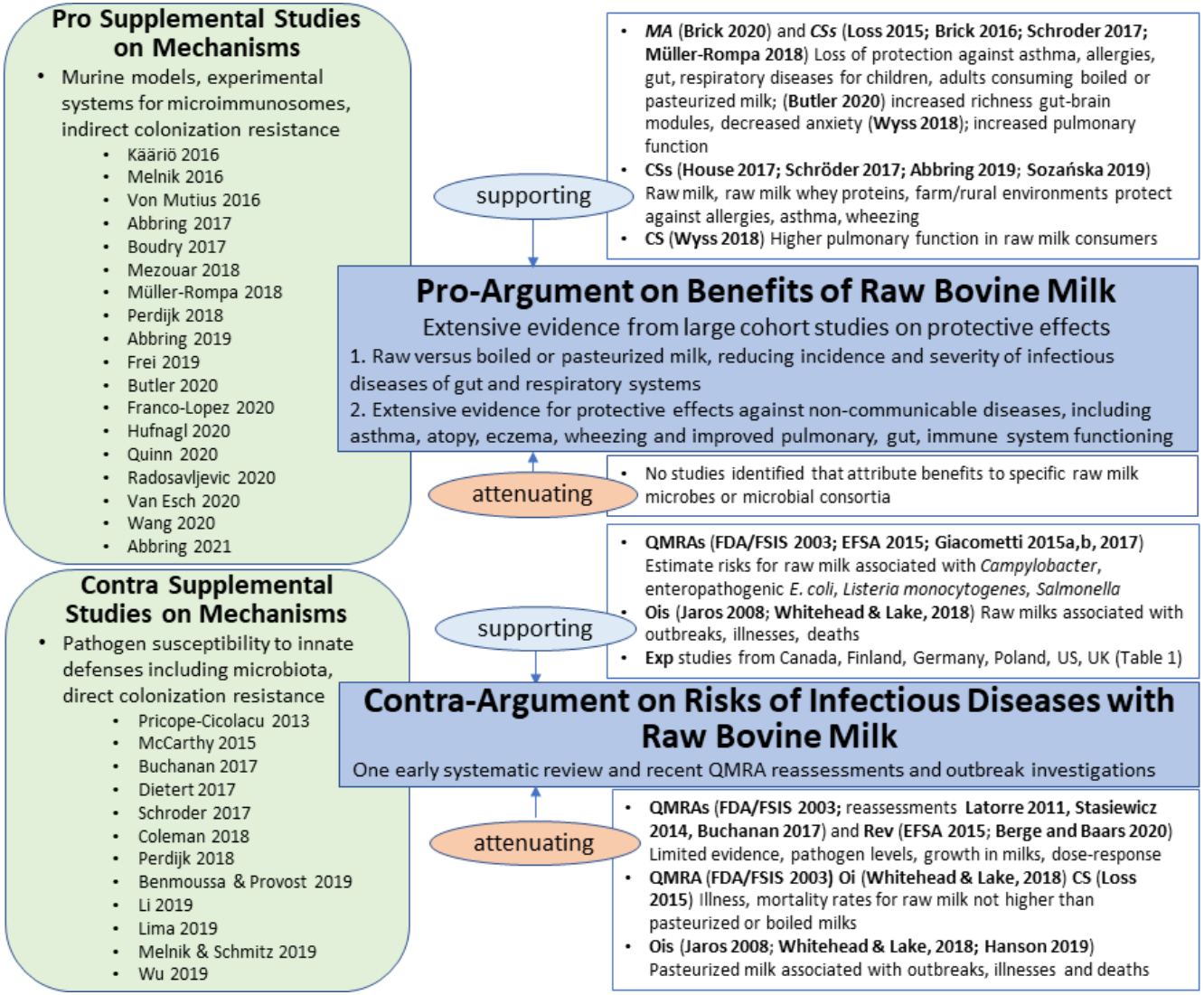
**21st Century Evidence on the Benefit/Risk of Raw Cow's Milk: Factoring in the Microimmunosome and Risk of NCDs**

Microbiota-Laden Complete Food
Benefit to the Microimmunosome
Reduced NCD Risk




Risk from Pathobionts
in Light of Modern-Day
Practices

# Evidence Map for Bovine Milk Ecosystem




## Evidence Basis

- 1 **MA;** 9 ***CSs*** including 1 experimental ***DBHPP;*** 7 ***QMRAs;*** 3 ***Ois*** including 1 ***SR;*** 2 **Rev,** 9 **Exp:** Cite consistent evidence
- 30 supplemental studies on plausible mechanisms for effects

## Conclusions

1. Overall biological benefits associated with raw milk consistent across multiple large ***CSs*** and a ***DBHPP***, with supplemental studies on plausible mechanisms
2. Limited evidence for milk-borne risks of infectious diseases in children and adults for both raw and pasteurized milks

## Remaining Uncertainties

- Are levels of pathogens in raw milk (and milk microbiota) predictive of risks (and benefits)?
- Is risk to children higher than adults based on current evidence and analysis?
- Is 'zero tolerance' for pathogens in raw milk scientifically, economically, ethically defensible?
- Who benefits from access to raw and pasteurized milks?
- What level of risk reductions are achievable for HACCP, cold chain, other?

# Cohort of 983 Children

## Raw Milk Protects Against Common Respiratory Diseases

1. Consumption of breastmilk and raw cow milk provided **comparable protective effects** against **respiratory and other infections**

2. Controlling for breastfeeding, **raw cow milk** consumption provided **protective effects** against:
   - Rhinitis ($p=0.015$)
   - Respiratory Tract Infections ($p=0.045$)
   - Otis ($p<0.001$)
   - Fever ($p=0.058$)

3. Commercial pasteurized milk was protective against fever, and Ultra High Temperature (UHT) milk and formula not protective against infections

4. **No clear associations reported for diarrhea and milk consumption**

**Loss** et al., **2015**. Consumption of unprocessed cow's milk protects infants from common respiratory infections. *Journal Allergy and Clinical Immunology*

# Pilot Study in 11 Allergic Children

## Reduced Allergenicity for Raw Milk; Likely Mechanisms

**TABLE 1** Organic raw cow's milk tolerated by cow's milk allergic children

| | | | Skin | | Serum | | DBPCT | |
|---|---|---|---|---|---|---|---|---|
| Patient | Gender | Age (y) | SPT (mm) | APT (class) | Total IgE (kU/L) | Specific IgE (kU/L) | Raw milk (mL) | Shop milk (mL) |
| 1 | M | 2.65 | 10 | ++ | 322.0 | 26.3 | 50.0 | 2.0 |
| 2 | M | 3.52 | 4 | ++ | 123.0 | 4.2 | 50.0 | 10.0 |
| 3 | M | 0.55 | 7 | +++ | 37.5 | 8.4 | 50.0 | 0.5 |
| 4 | F | 0.96 | 12 | ++ | 66.8 | 5.6 | 50.0 | 50.0 |
| 5 | M | 1.59 | 3 | +++ | nd | nd | 50.0 | 1.0 |
| 6[a] | M | 1.65 | 0 | + | nd | nd | 50.0 | 50.0 |
| 7[a] | M | 1.09 | 0 | + | nd | nd | 50.0 | 50.0 |
| 8 | M | 0.96 | 5 | ++ | 98.6 | 12.4 | 50.0 | 0.5 |
| 9 | F | 0.83 | 7 | +++ | 44.2 | 5.5 | 50.0 | 10.0 |
| 10 | F | 1.28 | 4 | ++ | nd | nd | 50.0 | 2.5 |
| 11 | M | 1.10 | 8 | +++ | nd | nd | 50.0 | 1.0 |
| Mean | | 1.49 | 6.7 | 2.4 | 115.4 | 10.4 | 50.0 | 8.6** |
| SEM | | 0.32 | 1.0 | 0.2 | 43.4 | 3.4 | 0.0 | 5.3 |

*Note*: Shown are gender, age, skin prick test, atopy patch test and serum total and cow's milk-specific IgE levels of 11 cow's milk allergic children before oral provocation as well as their level of tolerance to organic raw cow's milk and conventional shop milk during oral provocation.

**Abbring** et al., **2019**. Milk processing increases the allergenicity of cow's milk—Preclinical evidence supported by a human proof-of-concept provocation pilot.
*Clinical Experimental Allergy*

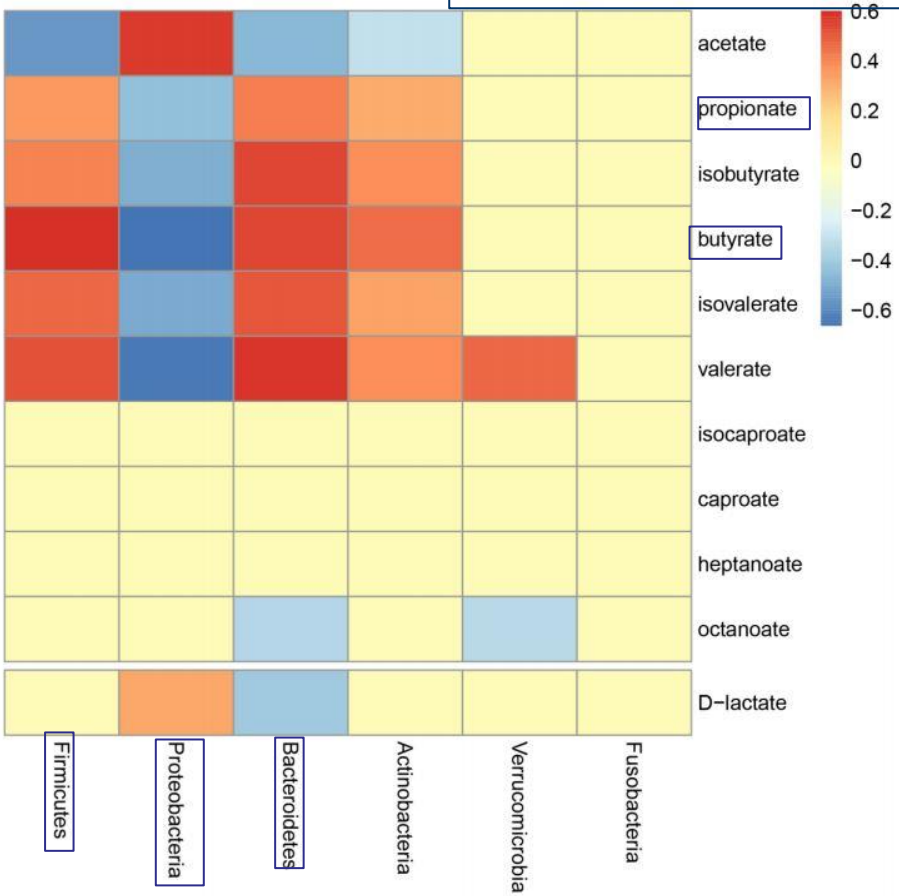
# Function of Gut Microbiota in Children: Correlations of Phyla with Levels of SCFAs, Lactic Acid

**Short Chain Fatty Acids (e.g., butyrate, propionate)**

**Children with intestinal failure (IF)**
- significantly less propionic and butyric acid
- significantly more D and L-lactate

**Underappreciated corollary:**
- Broadly diverse functional redundancy for gut bacteria producing SCFAs

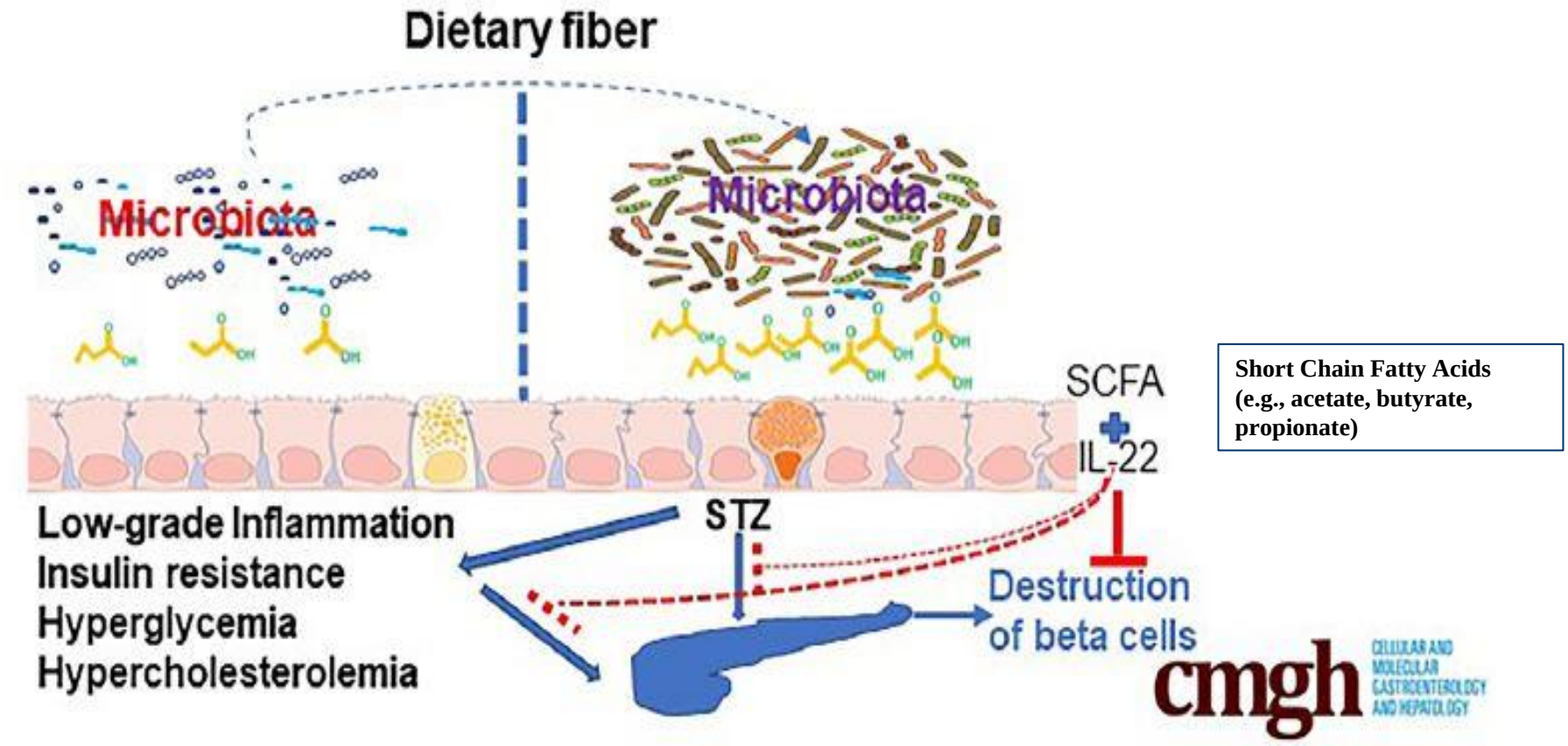
**One strategy** for **'managing our microbes'** might be to replace antibiotic administration with daily doses of a **Synbiotic**
- prebiotic nutrients for SCFA-producers PLUS
- probiotic strains that effectively metabolize those prebiotics in the gut




**Supplementary Figure 2.** Heatmap of correlations between the main 6 phyla of the gut microbiota and short-chain fatty acids and D-lactate (both per gram dry feces).

**Neelis** et al., **2021**. Gut microbiota and its diet-related activity in children with IF on long-term parenteral nutrition. *Journal Parenteral Enteral Nutrition*

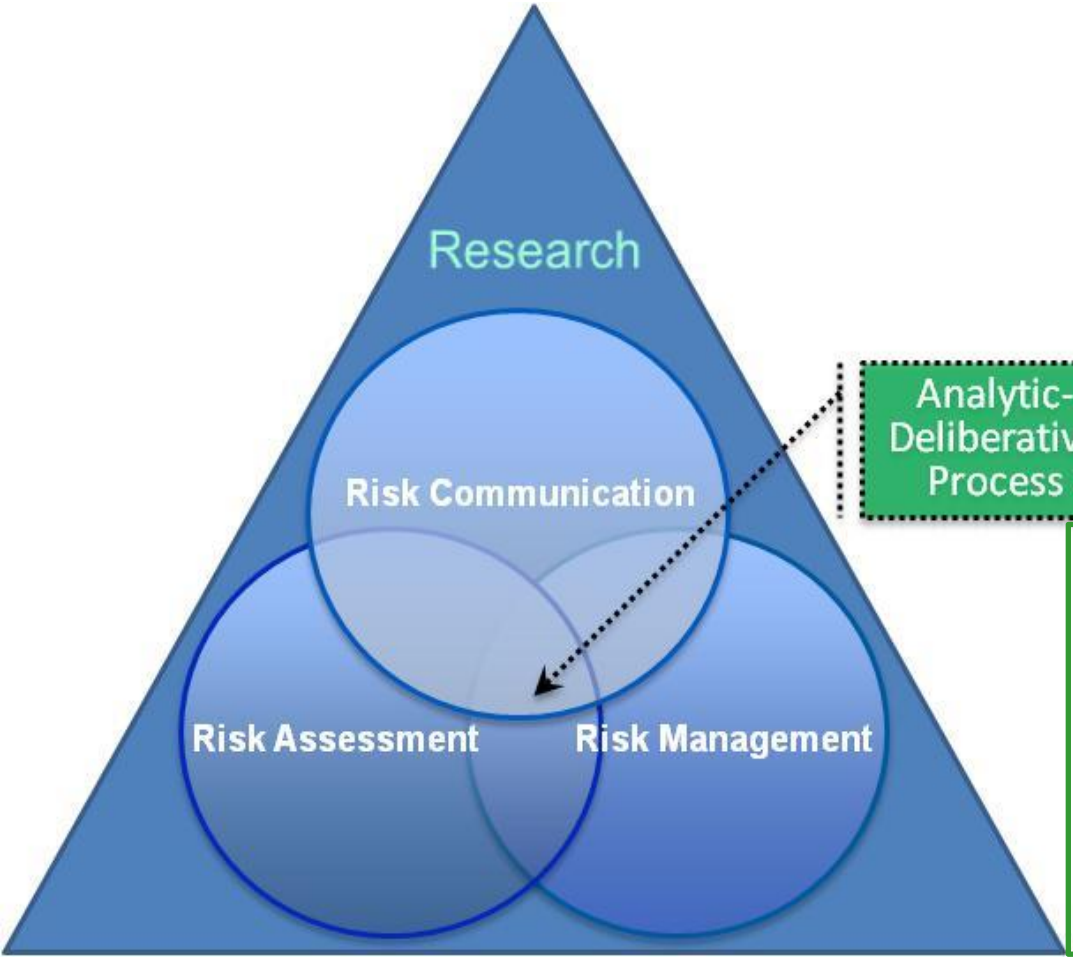
# Gut Microbiota, Dietary Fiber, Inflammation, Metabolic Diseases



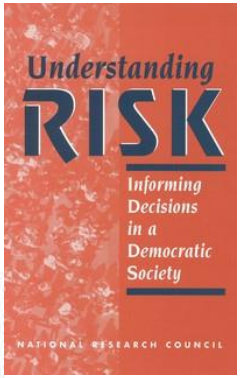

**Short Chain Fatty Acids (e.g., acetate, butyrate, propionate)**

**Zou** et al., **2021**. **Inulin** fermentable fiber **ameliorates type I diabetes** via IL-22 and **SCFAs** in experimental models. *Cellular and Molecular Gastroenterology and Hepatology*

# 21st Century Science Reveals Risks & Benefits to Microbes, including Pathogens




Analytic-Deliberative Process




*Free download at National Academies Press https://www.nap.edu/catalog/5138/understanding-risk-informing-decisions-in-a-democratic-society*

Builds in cycles of **research, analysis**, **deliberation**, and **interpretation** with stakeholders on

- **what goes in** (data, assumptions)

**AND**

- **what comes out** of risk models (**estimates of risk, uncertainty**).

*When perceptions of risk don't match up, need analytic-deliberative process.*

# Context for Analytic-Deliberative Process

**Problem:** **conflicting risk perceptions** in the media lack transparency about the **supporting scientific evidence**, impeding effective collaborative public discourse and misleading consumers and regulators.

- **Emerging science for SARS-CoV-2 pandemic**

**Desired Outcome:** develop reasoned, coherent, evidence-based regulations, policies, and communications that **balance benefits and risks**

## Barriers:

- **Outdated QMRA paradigms** exclude significant 21st century advances in scientific knowledge about the human '**superorganism**' and food microbiota that influence health and disease
- **Debates/uncertainties may fuel** rather than **resolve conflict** because debaters may reason from selected studies and not the full body of scientific evidence
- **Fear of microbes** as **germs** that will kill us (**germophobia**) overemphasizes risks

# Overall Summary

1. Incorporating **21st century science and analysis,** including data on ecosystems, **microbiota** of non-sterile **foods** and **gut microbiota**, and **microimmunosomes**, is greatly advancing capabilities to predict (and manage) severe disease, often in real time

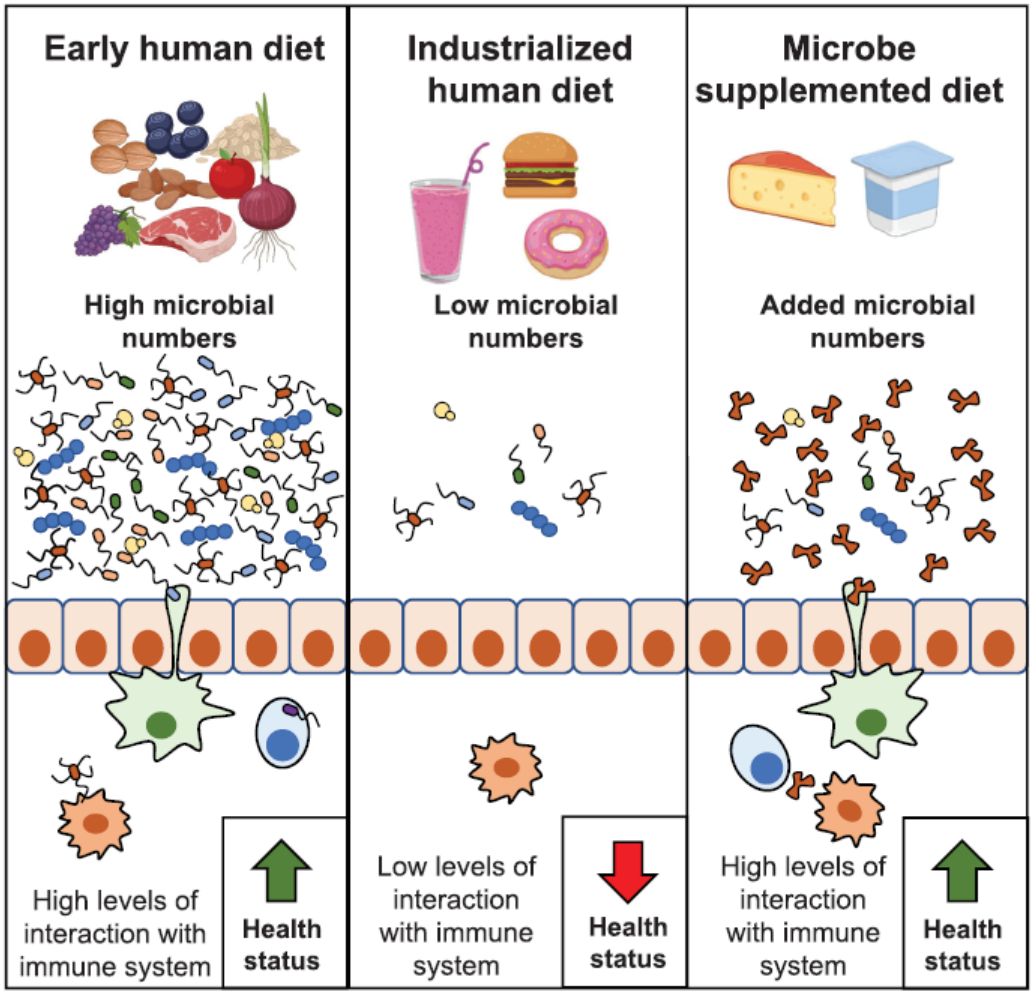
2. Many **risk practitioners** acknowledge the need for **quality risk analysis** and **investment in analytic-deliberative processes** with stakeholders, especially when **perceptions** and **estimates** of risks and benefits **don't add up** for **controversial global problems**

3. Just as for chemical risk assessment, **compounding conservatism** problems (**biased growth models, low-dose-linear non-threshold dose-response models** without effects of **immunity** and **vaccination**) warrant analytic-deliberative process

4. Any **student**, **faculty**, and others seeking to contribute to **stimulating trans-disciplinary collaborations** on **risks and benefits** can **join Society for Risk Analysis (SRA)** and its **regional organizations** including Upstate NY SRA

# Consider *Recommended Daily Allowances for Microbes* ($RDA_M$) as for Vitamins

(Hill, 2018)




(Marco et al., 2020)

**Whole Foods Contribute More than Nutrients for Human Cells**


http://www.sra.org/upstateny/

# Questions?


Email: colemanmellen@gmail.com

# Backup Slides on Microbial Risks

# Suggested Reading

## Margaret E. (Peg) Coleman and Colleagues, *Risk Analysis*, *Applied Microbiology*, and More

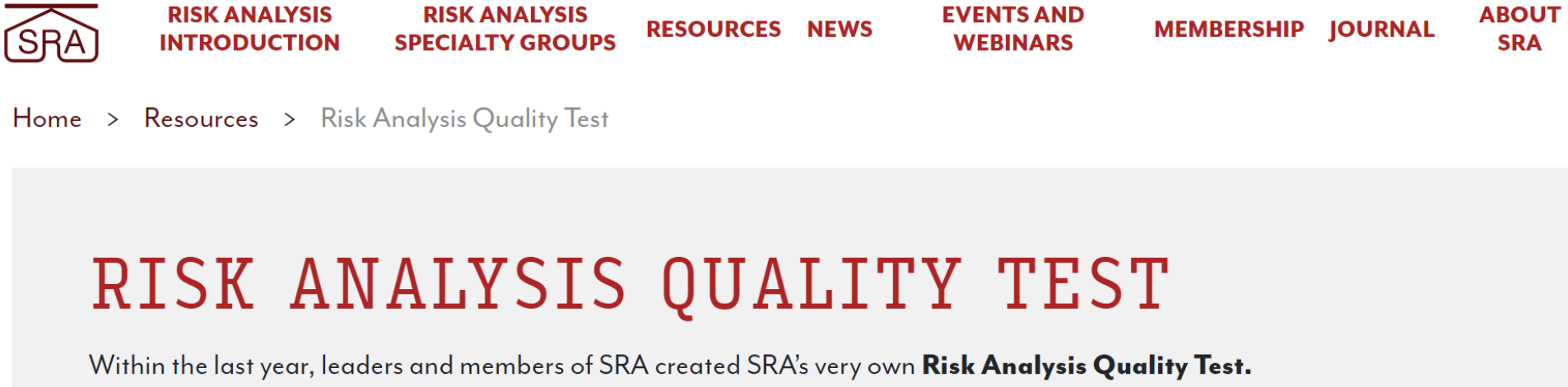

- **1996**: National Research Council (NRC) Understanding Risk: Informing Decisions in a Democratic Society.
- **1998**: Marks, H.M.; Coleman, M.E.; Lin, C.T.J. Topics in Microbial Risk Assessment: Dynamic Flow Tree Process. Risk Anal. 18, 309–328.
- **2011**: Wiedemann, P.; Schütz, H.; Spangenberg, A.; Krug, H.F. Evidence Maps: Communicating Risk Assessments in Societal Controversies: The Case of Engineered Nanoparticles. Risk Anal. 31, 1770–1783.
- **2018**: Coleman, M., Elkins, C., Gutting, B., Mongodin, E., Solano-Aguilar, G., Walls, I. Microbiota and Dose Response: Evolving Paradigm of Health Triangle. Risk Anal. 38, 2013–2028.
- **2020**: North, D.W. Risk Analysis, Decision analysis, causal analysis, and economics: A personal perspective from more than 40 years experience. Risk Anal. 40, 2178–2190.
- **2021a**: Coleman, M.E., North, D.W., Dietert, R.R., Stephenson, M.M. Examining Evidence of Benefits and Risks for Pasteurizing Donor Breastmilk. Applied Microbiology 1(3):408-425. https://doi.org/10.3390/applmicrobiol1030027.
- **2021b**: Coleman, M.E., Dietert, R.R., North, D.W., Stephenson, M.M. Enhancing Human Superorganism Ecosystem Resilience by Holistically 'Managing Our Microbes'. Applied Microbiology. 1(3): 471-497. https://doi.org/10.3390/applmicrobiol1030031 .
- **2022**. Dietert, R.R., Coleman, M.E., North, D.W., Stephenson, M.M. Nourishing the Human Holobiont to Reduce the Risk of Non-Communicable Diseases: A Cow's Milk Evidence Map Example. Applied Microbiology. 2(1):25-52. https://doi.org/10.3390/applmicrobiol2010003.
- **2022**. North, D.W., Coleman, M.E., Hull, R.R. Need for International Workshops to Deliberate Evidence of Benefits and Risks of Raw Milks. Accepted in Corpus Journal of Dairy and Veterinary Science.


## Rodney R. Dietert, Collaborator and Emeritus Professor of Immunotoxicology, Cornell University


- **2016**: Dietert, R.R. The Human Superorganism: How the Microbiome Is Revolutionizing the Pursuit of a Healthy Life; Dutton,NY.
- **2015**: Dietert, R.R.; Silbergeld, E.K. Biomarkers for the 21st Century: Listening to the Microbiome. Toxicol. Sci. Off. J. Soc. Toxicol. 144, 208–216.
- **2017**: Dietert, R.R. Safety and Risk Assessment for the Human Superorganism. Hum. Ecol. Risk Assess. 23, 1819–1829.
- **2018**: Dietert, R.R. A Focus on Microbiome Completeness and Optimized Colonization Resistance in Neonatology. NeoReviews 19, 78–88.
- **2021**: Dietert, R.R.; Dietert, J.M. Twentieth Century Dogmas Prevent Sustainable Healthcare. Am. J. Biomed. Sci. Res. 13, 409–417.

# SRA Resources and Tools

**(https://www.sra.org/)**




1. https://www.sra.org/resources/risk-analysis-quality-test/
2. https://www.sra.org/resources/students-young-professionals/
3. https://www.sra.org/webinar/being-a-young-risk-scientist/
4. https://www.sra.org/resources/specialty-risk-resources/
5. https://www.sra.org/resources/covid-19-resources/

# Risk Management and the Wisdom of Aldo Leopold

(Warren and Kieffer, 2010, *Risk Analysis* 30(2))

## Aldo Leopold

- Ecologist, conservationalist, author of *A Sand County Almanac*
- Biotic or land pyramid of energy circuits, food webs, ecological interdependencies: complex functional interactions of climate and atmosphere, rocks, soils, waters, plants, animals, {microbes}, operating together as an interdependent communities, complex ecosystems
- Warren and Kieffer applied Leopold's body of work as criteria for contemporary risk management

## Criteria of 'Land Health' Motivated by Land Ethic

- Common technology-driven over-consumptive lifestyle in US and other developed countries disconnected from natural systems of planet Earth and possibilities for just, prosperous, enduring, and peaceful global civilizations

## 'Stealth Disasters' Caused or Amplified by Human Activity

- 1930s Great Plains Dust Bowl crisis, combination storms, drought, agriculture methods not attuned to protecting soils in dry windy climate
- Industrialized agricultural monocultures with chronic loss of soil fertility and biodiversity in conventionally plowed, fertilized, and irrigated agricultural systems
- Loss of biodiversity, plants, animals, microbes, … diminishes nature's capacity for self-organization, self-renewal, self-healing, resilience
- Disproportionate impacts to poor, marginalized, undervalued when ecology is too simplistic or rudimentary

## Call for scientists to communicate understanding of natural (and disturbed) systems to conserve land with its biota AND reduce risks to ecosystems, humans and natural environment

- Concern about ecology and risk management from both scientific and cultural or philosophical perspectives
- Avoid diminishing nature's capacity for self-renewal (translation: avoid 'stealth disasters'; promote 'regenerative agriculture')

# Risk Management Solution: Remove HIGH CONCENTRATIONS of Environmental Microbes

**Source**: *Vibrio cholerae*-contaminated drinking water near cesspits in industrialized 19th century London

**Simple Solution**:

John Snow advised **removing the handle** on London water pump **near cesspit** that caused clusters of fatal cholera cases

- Removed **highly contaminated water** from drinking supply
- Scientific knowledge of **importance of sanitation** informed control of outbreaks

Sherman, 2007; http://www.csiss.org/classics/content/8

# Risk Management Solution: Remove HIGH CONCENTRATIONS of Environmental Microbes

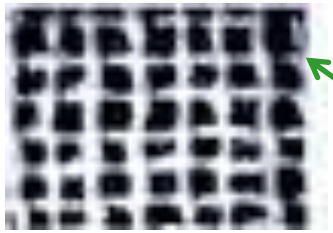
**Source:** *Vibrio cholerae*-contaminated surface waters in 21st century developing countries

**Simple Solution**:

Rita Colwell & Anwar Huq trained villagers to filter river water with common **cloth** (sari cloth)

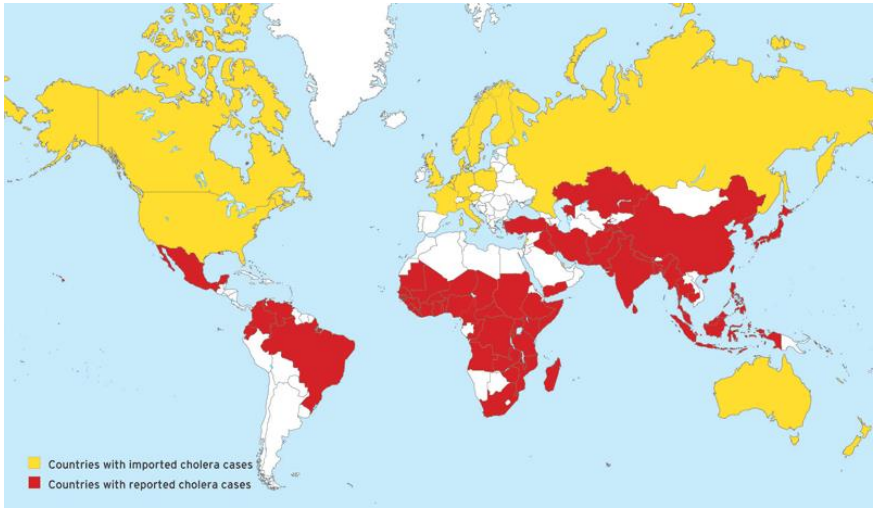
- Removed copepods that **concentrate** bacteria to **high doses**
- Scientific knowledge of **ecological link** of **copepods** and **cholera outbreaks** informed solution

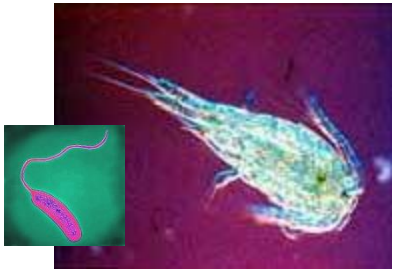
Colwell et al., 2003; Huq et al., 2005



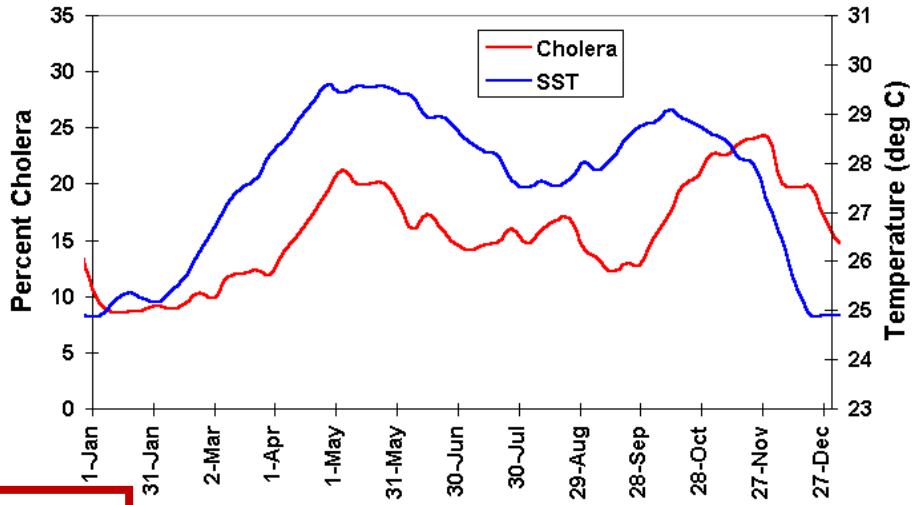
cloth filters **copopods,** NOT *Vibrio* bacteria

# Remote Sensing as Predictive Tool for Bacteria in Surface Waters???



- Surveillance at microscopic level **unnecessary** for cholera
- Remote sensing of **plankton blooms** and **water temperature** sufficient due to concentration of *V. cholerae* in copepods




Knowledge of ecosystem interactions enables testing at resolution appropriate to protect health

# Microbiomes of Natural and Built Environments: Subways

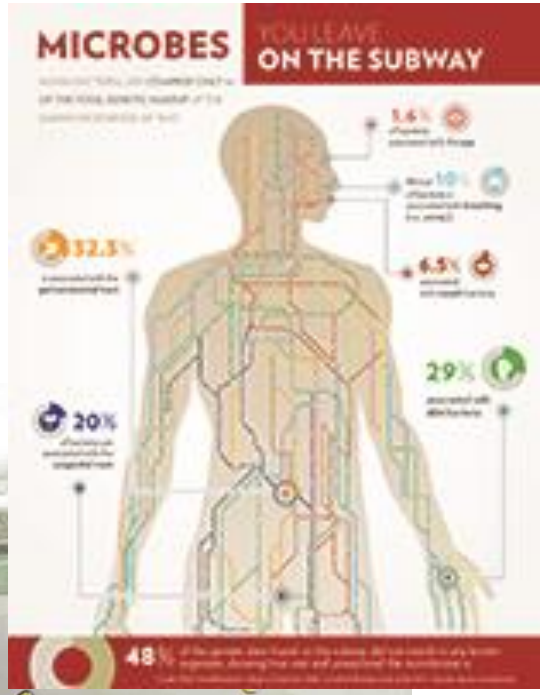
**Science News** *from research organizations*

## Mapping the subway's microbiome

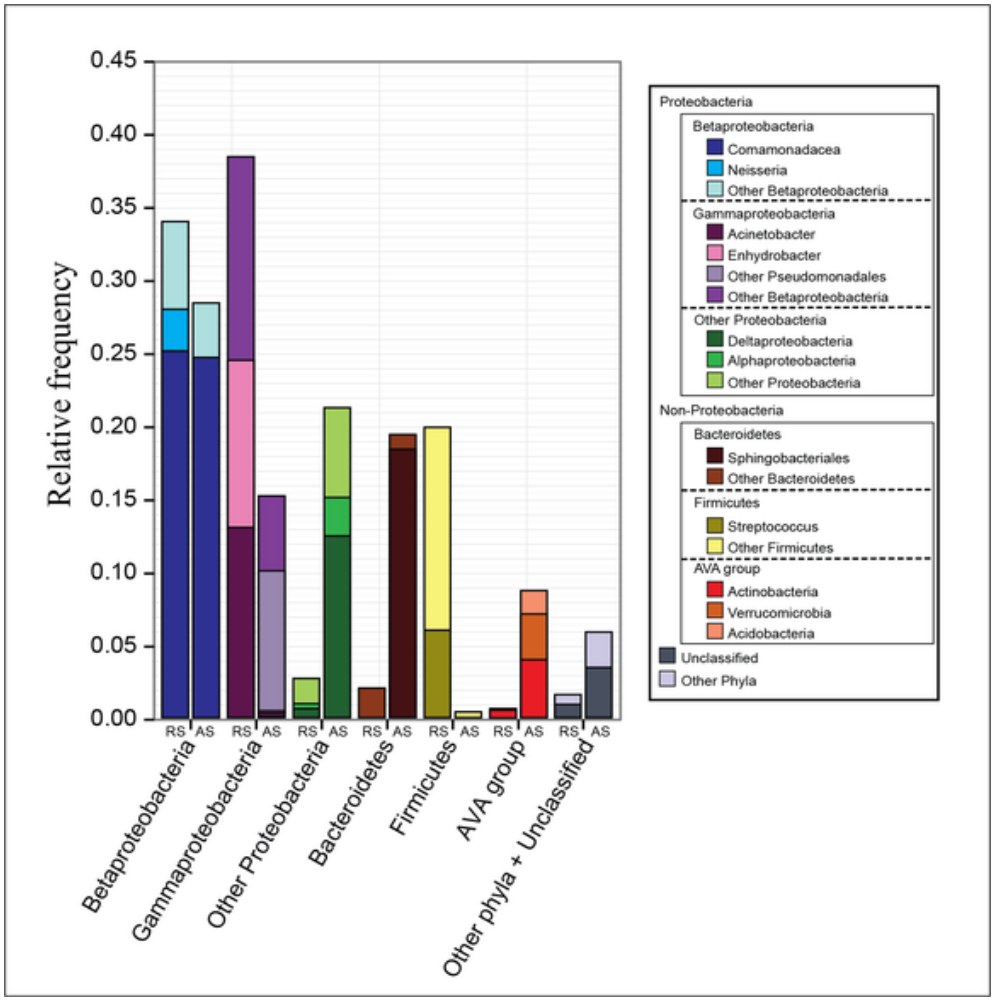
*Date:* June 21, 2016

*Source:* Centre for Genomic Regulation

*Summary:* Researchers aims to map the microbiome of public transit systems in 54 cities worldwide, including New York, Hong Kong, Paris or Sydney.



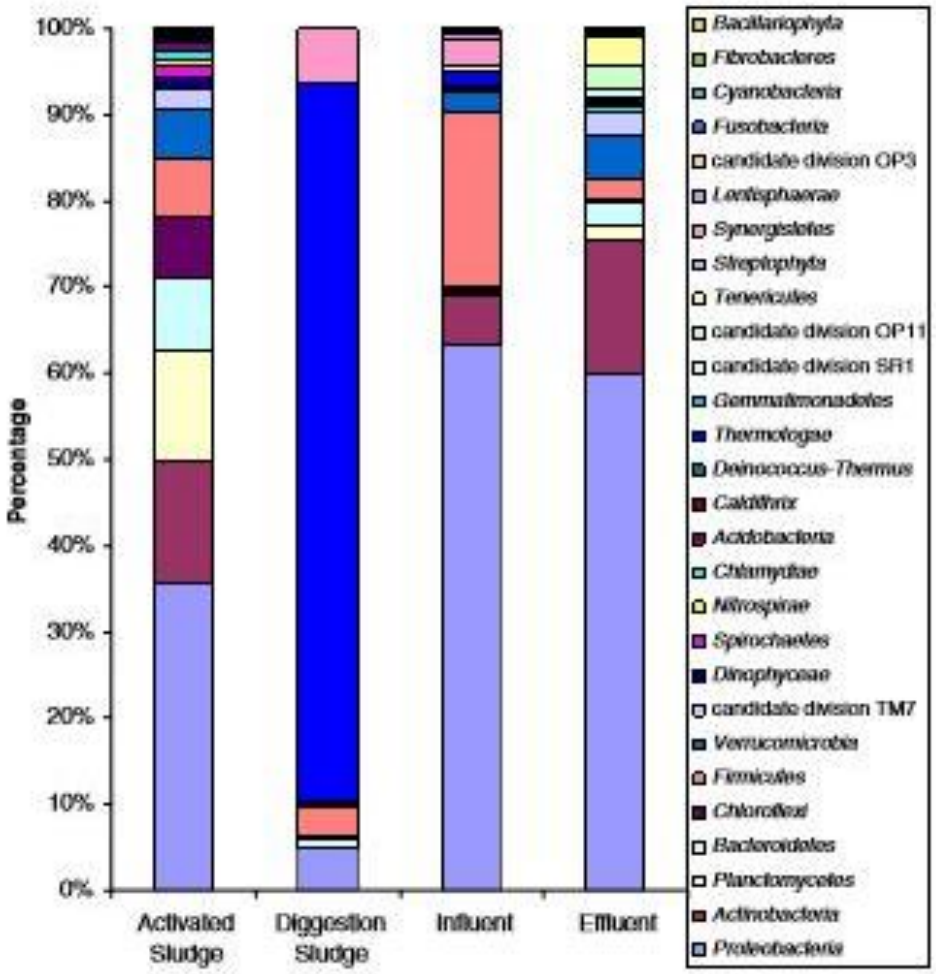
# Microbiomes of Natural and Built Environments: Sewage Treatment




Paiva MC, Ávila MP, Reis MP, Costa PS, Nardi RMD, et al. (2015) The Microbiota and Abundance of the Class 1 Integron-Integrase Gene in Tropical Sewage Treatment Plant Influent and Activated Sludge. PLOS ONE 10(6): e0131532. doi:10.1371/journal.pone.0131532
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0131532

PLOS ONE | TENTH ANNIVERSARY

# Microbiomes of Wastewaters




Bacterial Communities in Different Sections of a Municipal Wastewater Treatment Plant Revealed by 16S rDNA 454 Pyrosequencing. (Ye and Zhang, 2013)

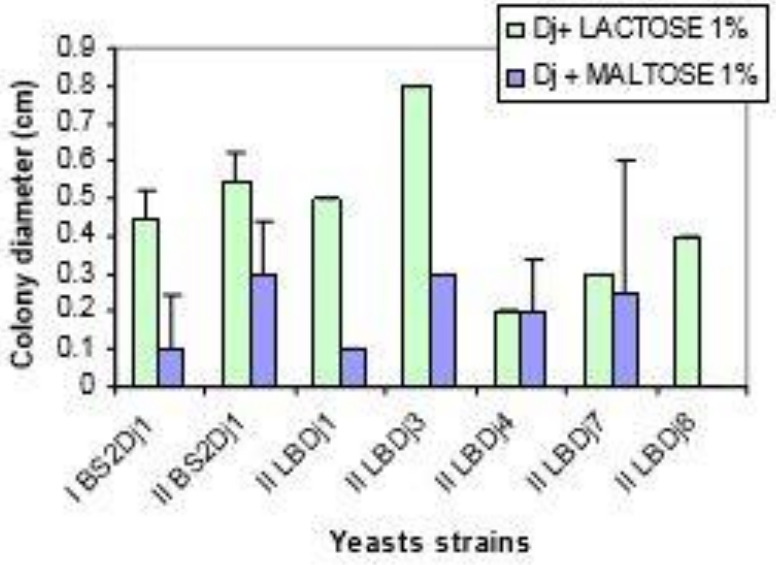
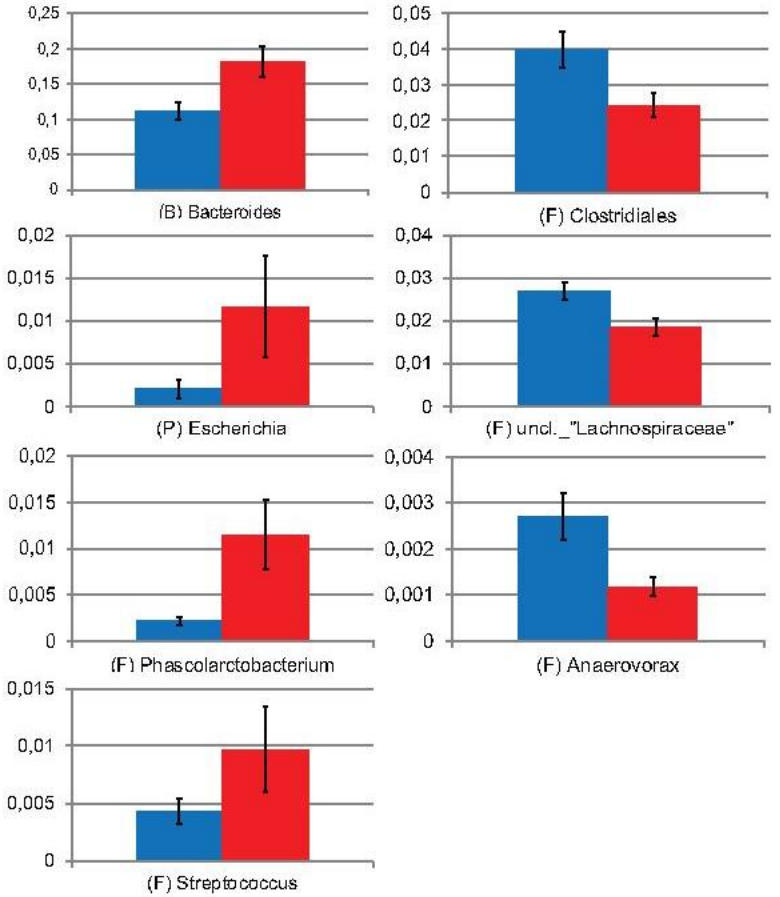



*Figure 7.* Food industry wastewater microbiota capacity to metabolise the simple glucides

Microbiological and Biochemical Characterisation of Dairy and Brewery Wastewater Microbiota (Palela, Ifrim and Bahrim, 2015)

# Microbiomes of Workers in Built Environments: Poultry Abattoirs

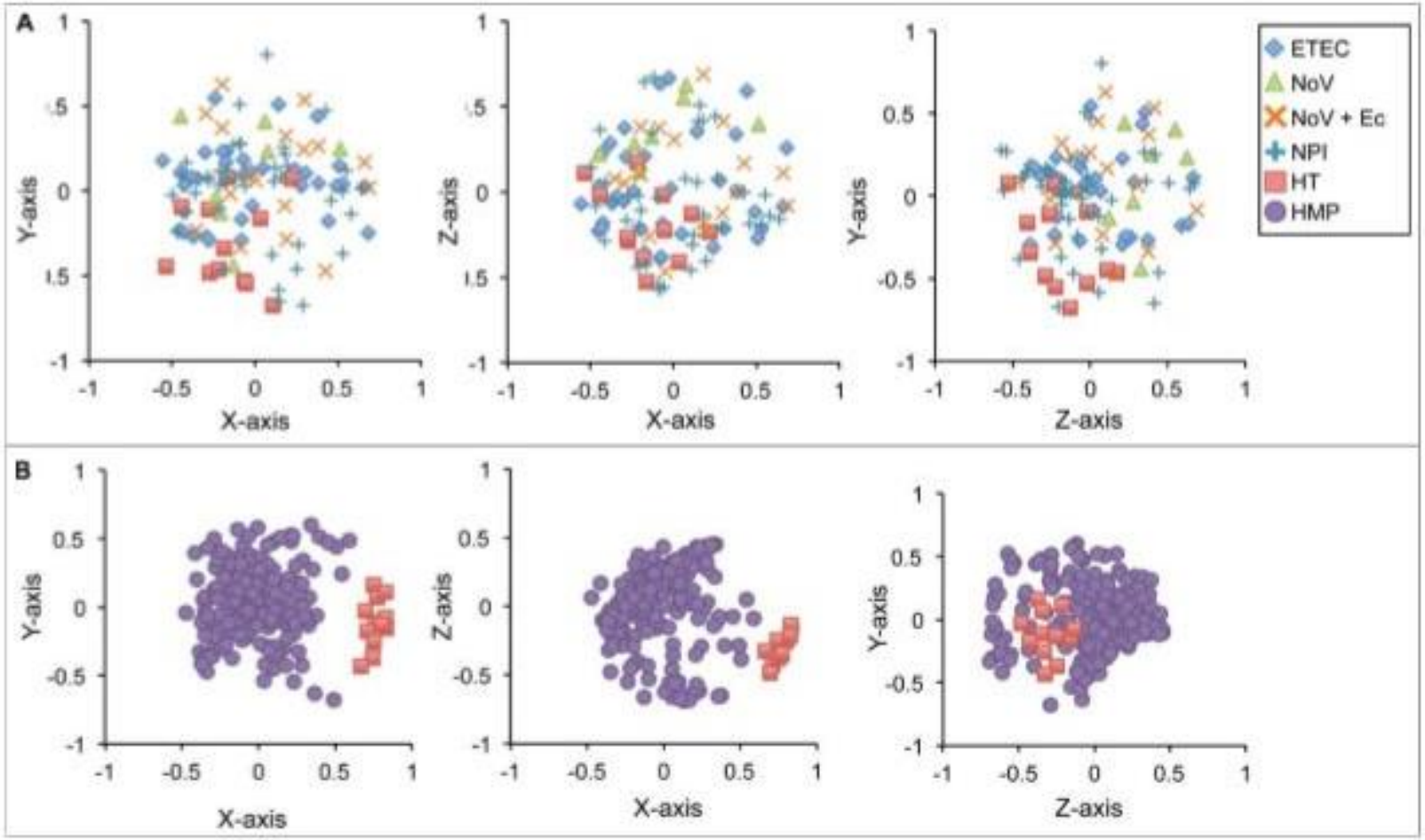
- Gut microbiota (N=24 poultry abattoir workers) monitored during peak *Campylobacter* exposures (all likely exposed)
- Culture-positive for pathogen (**red**)
  6 of 7 asymptomatic, significant long-term changes in gut microbiome
- Culture-negative for pathogen (**blue**)
  - significantly lower abundance of *Bacteriodes, Escherichia, Phascolarctobacterium,* and *Streptococcus*
  - significantly higher abundance *Clostridiales, Lachnospiraceae,* and *Anaerovorax*
- Frequent exposures can provide **colonization resistance** and protection from illness




Susceptibility to *Campylobacter* Infection Is Associated with the Species Composition of the Human Fecal Microbiota

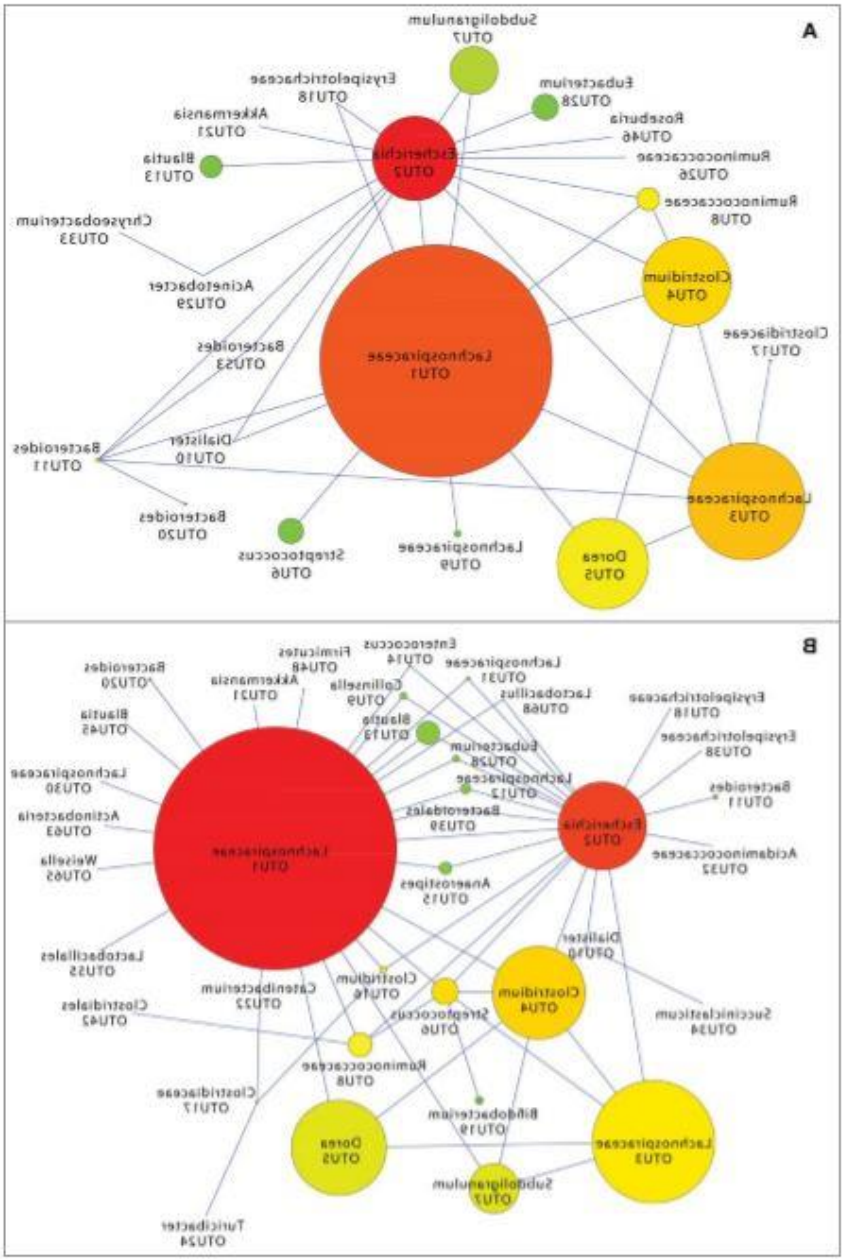
Johan Dicksved,[a,b] Patrik Ellström,[a] Lars Engstrand,[c] Hilpi Rautelin[a,d] 2014

# Community Structures: Healthy and Diseased




**Youmans et al., 2015.** Characterization of the human gut microbiome during travelers' diarrhea

# Community Structures: Healthy and Diseased




Characterization of the human gut microbiome during travelers' diarrhea

Bonnie P Youmans[1,#], Nadim J Ajami[1,2], Zhi-Dong Jiang[3], Frederick Campbell[4], W Duncan Wadsworth[4], Joseph F Petrosino[1,2], Herbert L DuPont[3,5,6,7], and Sarah K Highlander[8,*] 2015

# Childhood and Travelers' Diarrhea in Developing Countries

## Bacterial Pathogens:

- *Campylobacter*
- Entero-Toxigenic *E. coli* (ETEC)
- Entero-Pathogenic *E. coli* (EPEC)
- *Salmonella*
- *Shigella*
- *Vibrio*

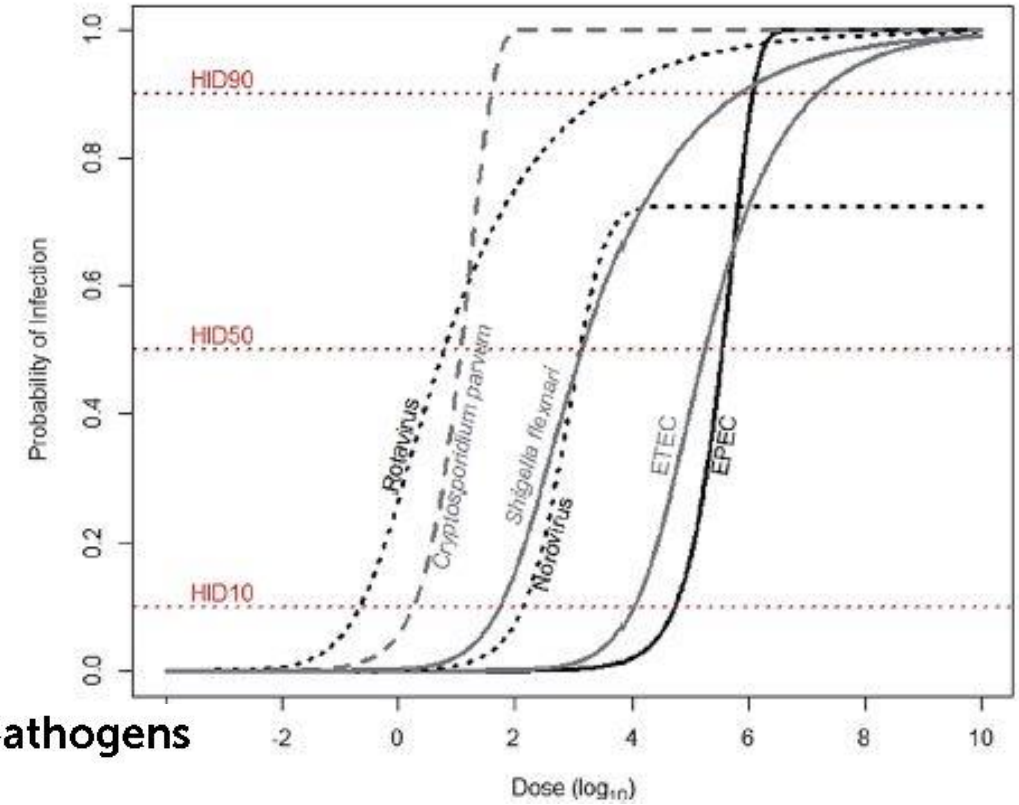
## Viral Pathogens:

- Norovirus, Rotavirus

Adults exposed to endemic pathogens develop resistance, but children and travelers susceptible.

# Childhood Diarrheal Morbidity (Mortality)

| | Effective Dose$_{50}$ |
|---|---|
| **1. ETEC** | $10^5$ to $10^8$ bacteria |
| **2. EPEC** | $10^5$ to $10^7$ bacteria |
| ***3. Shigella*** | $10^3$ bacteria |




**Environmental transmission of diarrheal pathogens in low and middle income countries**

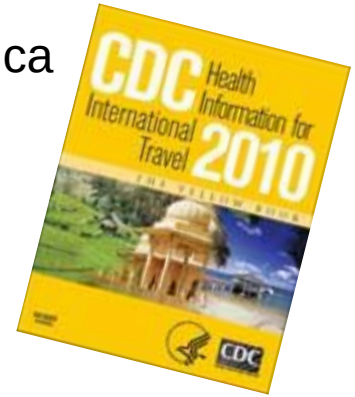
Timothy R. Julian 2017

Clinical trial with probiotic *E. coli* Nissle 1917 yielded significant reduction in childhood diarrhea duration (Henker et al., 2008)

# Predicting and Preventing Travelers' Diarrhea

International travelers to high and intermediate risk countries can be exposed to less controlled sanitation (more frequent and higher levels of contamination) in food and water that have caused 40-60% to contract travelers' diarrhea.

- **High risk**: Asia, Middle East, Africa, Mexico, Central/South America
- **Intermediate risk**: E Europe, S Africa, Caribbean islands
- **Low risk**: US, Canada, Australia, New Zealand, N/W EU




Resistant superorganisms?

**The Traveling Microbiome**

Mark S. Riddle[1] · Bradley A. Connor 2016

# Volunteers Administered *Campylobacter*: Study Host Risk Factors for Travelers' Diarrhea

Innate immunity for some volunteers after one high dose ($10^9$ or 1 billion bacteria)

Immunity from previous exposures

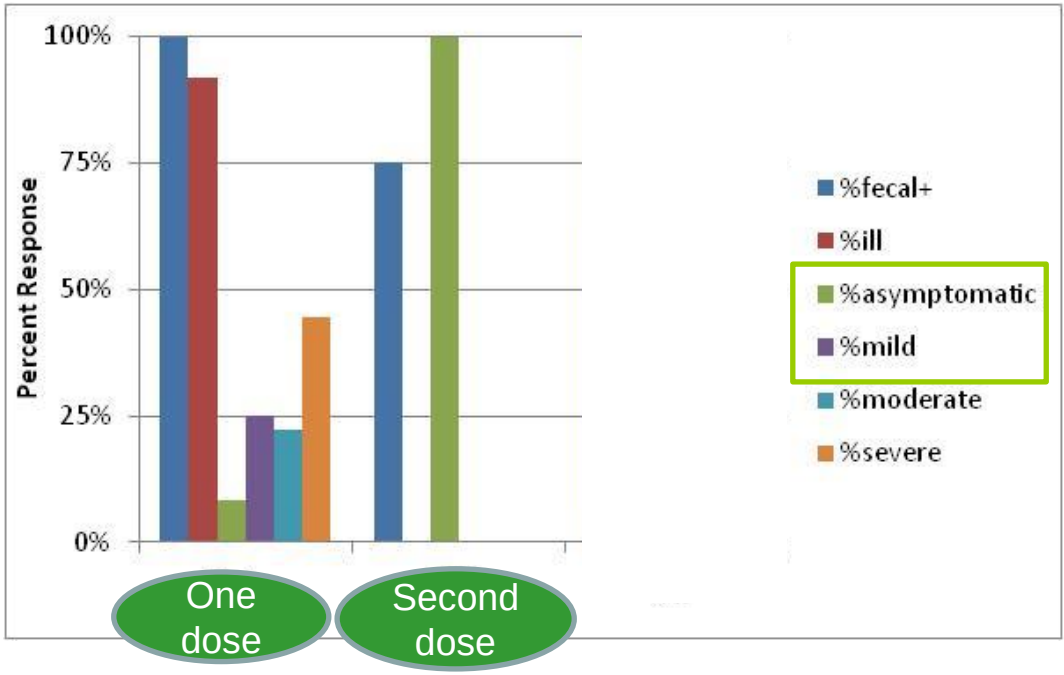
Fatigue and physical stress

Psychological stress

Boredom with ready-to-eat meals

Failure of **public health advice** to prevent travelers' diarrhea in soldiers deployed outside the US

Tribble et al., 2010



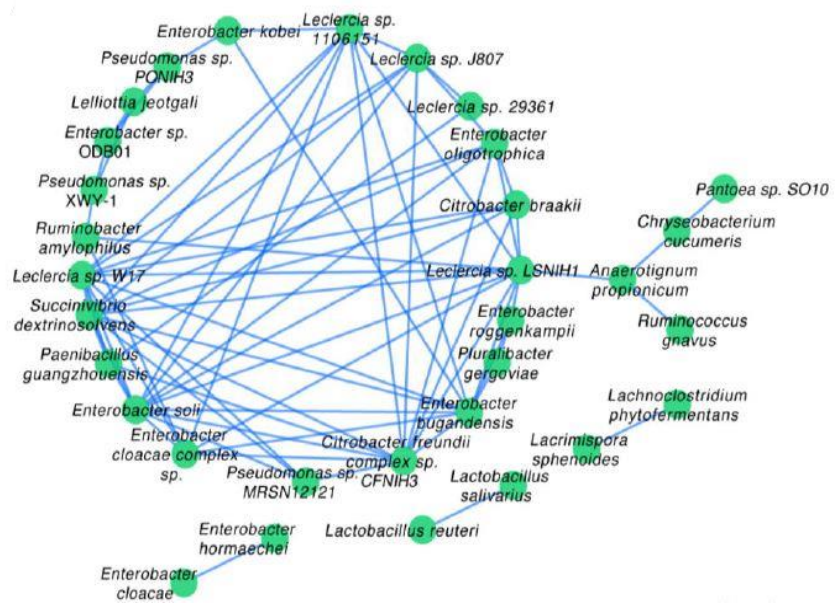

Avoid street vendor foods/beverages, raw and undercooked meat/seafood, raw fruits/vegetables, tap water, ice, unpasteurized dairy products

# Significant Differences in Lung Microbiota

## 23 Healthy Controls, 19 COVID Cases

- **lung bacteriome (99 species) + and - correlations**
- **lung virome (18 viruses)**

**Correlations** suggest **lung microbiota** merits further studies on **mechanisms** for interactions, temporal and spatial dynamics, and **causation** to identify priorities for **'managing our microbes'** in the respiratory tracts of COVID patients




**Han** et al., **2020**. The active lung microbiota landscape of COVID-19 patients. *medRxiv*

# ROOT CAUSES Fear and Dread of Microbes as Killer Germs; **Unquestioned Unstated Assumptions**, Speculations; Ideology and/or Politics Tipping Science

1. **The source of microbes in raw milks is feces**
   - Wu et al., 2019, 2022; Gomes et al., 2020

2. **Pasteurization is a 'silver bullet'**

3. **Pasteurized milk is zero risk**

4. **Raw milk is 'inherently dangerous'**

**RAQT Utility: Identify root causes (ideology, politics, and science) to enable future Evidence-Based Risk Management**

# Root Cause: Fear and Outrage about Raw Milk in US

Published peer-reviewed studies on next slide document urban 'swill milk stables' in and around large cities that contributed to high urban mortality for decades (**1840s to 1920s**)

https://www.brownstoner.com/history/walkabout-the-great-milk-wars-part-1/

- Unhealthy and dying cows in urban 'dairies', starved then fed hot brewery or distillery waste
- 'Swill milk' adulterated (added bicarbonate of soda, chalk, flour, plaster of Paris, salts, sugars, water) to mask thin bluish appearance
- 'Swill milk' recognized as contributor to high urban mortality, particularly infants and children
- Wealthy urban and rural families could buy or produce wholesome 'country milk' from healthy pasture raised cows

A diseased cow, unable to stand, is pulled up to be milked. Distilleries kept a stable of such animals, fed them mash and whiskey slops. The milk made babies tipsy and often sick.

- **Multiple contributing factors** for high urban mortality rates at turn of the 19th century as referenced in **Dietert** et al. (2022) and project bibliography

# Documentation of Sources Linked to Raw Milk Mortality

(Dietert et al., 2022)

High rates of urban vs rural mortality at the turn of 19th century attributed to multiple factors:

- Industrialization and urbanization (including dairies)
- Dangerous partnerships between distillers and urban dairies that persisted for decades
- **Urban populations** suffered **lack of**:
  - Safe water
  - Reliable systems of sewage and manure disposal
  - Reliable refrigeration during milk transport and in kitchens
  - Quality and quantity of foods for poor; undernourished, malnourished (wealthy could afford 'country milk' from pasture raised cows)
  - Healthy working conditions, adequate housing and medical care for the poor; fatigued (overcrowded, unventilated)

- Organizing Protest in the Changing City: Swill Milk and Social Activism in New York City, 1842–1864. (Egan, 2005)
- From Swill Milk to Certified Milk: Progress in Cow's Milk Quality in the 19th Century. (Obladen, 2014)
- Mortality Differentials between Rural and Urban Areas of States in the Northeastern United States 1890-1900. (Condran & Crimmins, 1980)
- Watersheds in Child Mortality: The Role of Effective Water and Sewerage Infrastructure, 1880 to 1920. (Alsan & Goldin, 2019)
- Regional and Racial Inequality in Infectious Disease Mortality in U.S. Cities, 1900-1948. (Feigenbaum et al., 2019)
- Mortality Variation in U.S. Cities in 1900: A Two-Level Explanation by Cause of Death and Underlying Factors. (Crimmins & Condran, 1983)