



Risk Beyond Numbers of Foodborne Illnesses: Understanding Data, Gaps, and Assumptions

**Presented by Peg Coleman on November 16, 2015
At the Wise Traditions Conference in Anaheim, CA**



Who we are

- Woman owned small business specializing in **medical microbiology** and scientific support for **microbial risks**

What we provide

- Analysis and training about safety of exposures to bacteria 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

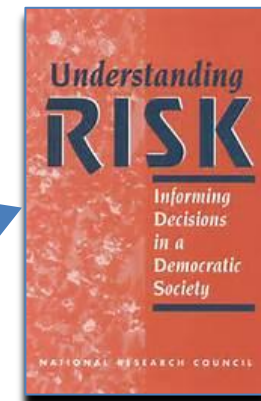
<http://www.colemanscientific.org/index.html>

Outline

- Risk Analysis: Living with Risk
- Principles and Practices of Risk Assessment
 - Exposure
 - Dose-Response
- Risk Perceptions and Estimates for Listeriosis in Milk
 - FDA Exposure
 - FDA Dose-Response
- Natural Microbiota Protects Against Pathogens
- Need for Open Public Dialogue
(Analytic-Deliberative Process)
- Key References

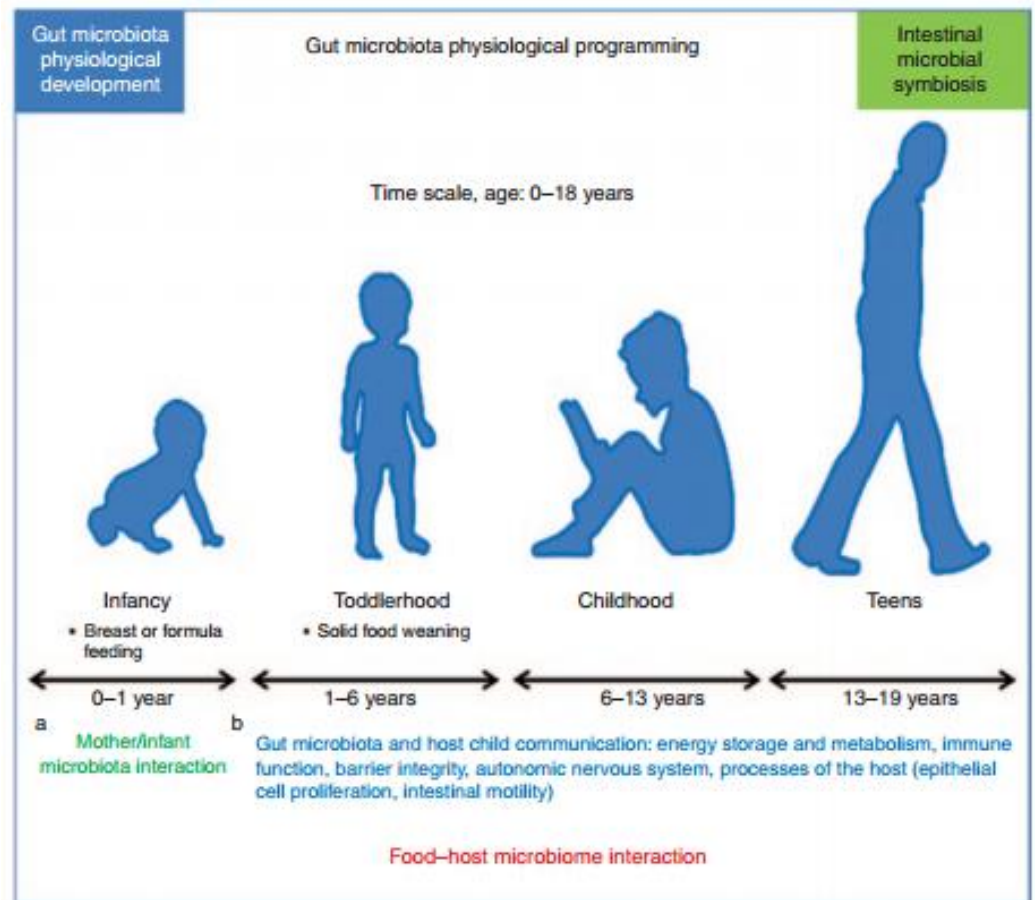


National
Academy of
Science



Introduction: Living with Risk

- **Bacteria are all around (and inside) us!**
- **Few** bacteria cause **disease (pathogens)**
- **Predicting risk** of illness is **complex** and **uncertain** (e.g., age, environment)
- **Perceptions of risk** in the media often not supported by **science**



Putignani, et. al., 2014, <http://www.nature.com/pr/journal/v76/n1/pdf/pr201449a.pdf>

Describing Risk



- **Threats to human health** from some bacteria in our environments (and in or on our bodies)
- **Uncertain consequences** of future event leading to illness

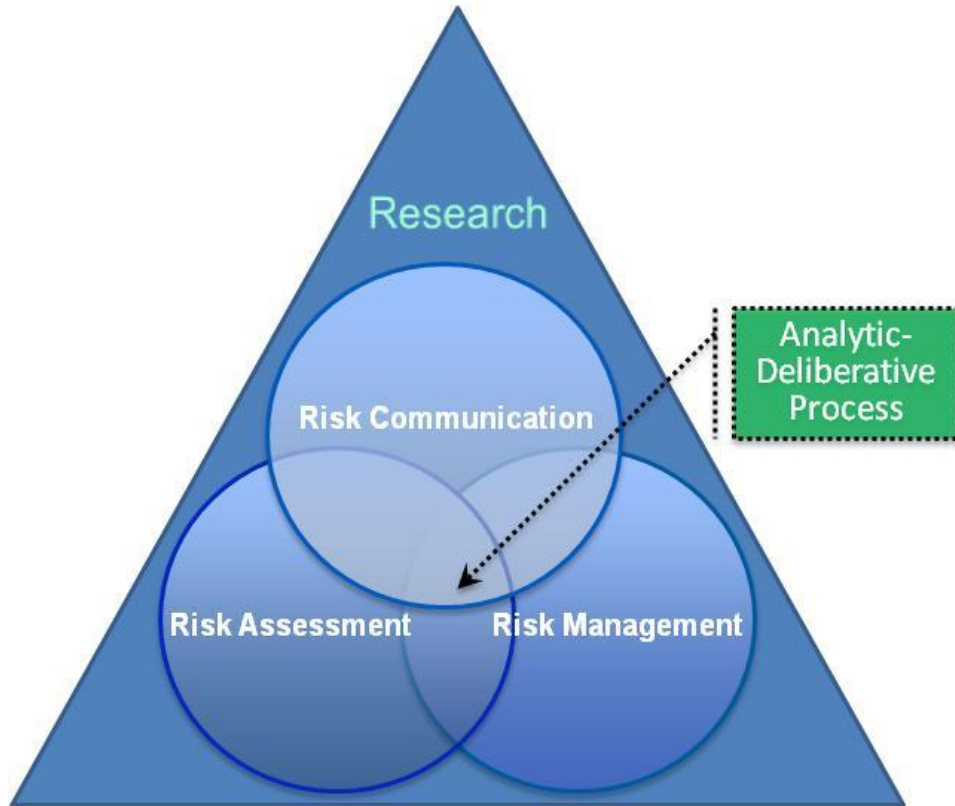
Many risk descriptions possible, but today's focus is on **risk of foodborne disease**

Risk Analysis

Requires **multiple disciplines** to **assess**, **communicate**, and **manage** risk in a **transparent interactive process** that involves **stakeholders** (consumers, interested parties with 'stake' in foodborne risk management).

- Risk **Assessment** (natural sciences – microbiology, mathematics)
- Risk **Communication** (social sciences – public discourse)
- Risk **Management** (decision sciences – cost/benefit analysis)

When Perceptions of Risk Don't Match Up



-
- Intended to **reconcile differences**
 - Builds in the cycles of **analysis**, **deliberation**, and **interpretation** with stakeholders on
 - **what goes in** (data, assumptions) **and**
 - **what comes out** of risk models (**estimates of risk, uncertainty**).
-

Challenging Myth with Science

Fact?

Possibility?

Fiction?



- Need **science** to challenge myths and urban legends
 - **Presence** of bacteria alone **insufficient** to predict **response** (illness, death)
 - **Dose** (amount) of **pathogenic bacteria ingested matters**
 - Risk assessors develop **dose - response** curves to **simulate** foodborne illness
- Need to improve public service and education
 - **Uncertainty** and **variability** in dose - response relationships **poorly explained**
 - Scientific **facts** not generated by risk simulations – need to enhance public understanding of model inputs (**science**) and outputs (**simulations**)

Bias in Risk Perceptions and Estimates

Objective studies including many from **National Academy of Sciences** panels challenge assumptions, bias, misconceptions, opinions, and oversimplifications in risk models



- Pathogens grow in foods (**sometimes true**)
- Pathogens grow in foods as in laboratory (**mostly false**)
- Exposure in foods alone predicts illness (**false**)
- Ingestion of single pathogen cells causes fatalities (**false, no 'superbugs'**)
- Natural repair and replacement of infected cells can't protect hosts from disease (**mostly false**)
- Other natural (**innate**) host defenses can't protect against disease in healthy humans (**mostly false**)



Examples from Diseases in the Media

- Anthrax (*Bacillus anthracis*) in Air
- Bloody diarrhea (*E. coli* O157:H7) in Ground Beef, Foods
- Campylobacteriosis (*Campylobacter* spp.) in Foods
- Cholera (*Vibrio cholerae*) in Water
- **Listeriosis** (*Listeria monocytogenes*) in Milk
- Salmonellosis (*Salmonella* spp.) in Foods
- Tularemia (*Francisella tularensis*) in Air and Water

Risk Triplet



Risk assessors define risk by answers to a triplet of questions:

Plain language

1. What can go wrong?
2. How likely is it?
3. What are the consequences?

Symbolic language

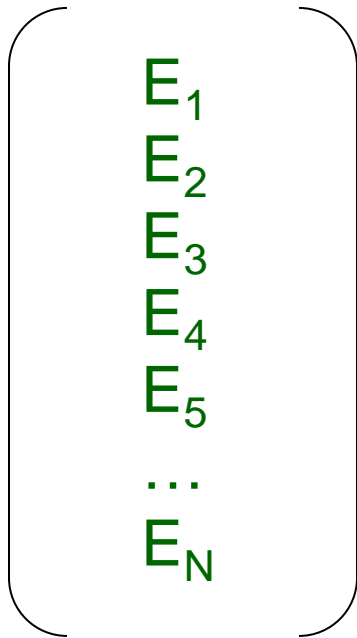
S_i , (set of scenarios)
 ℓ_i or $P_i(\varphi_i)$
 X_i or $P_i(X_i)$

$$\text{Risk} = \{S_i, P_i(\varphi_i), P_i(X_i)\}_C = \{S_0, P_0(\varphi_0), P_0(X_0)\} + \{S_i, P_i(\varphi_i), P_i(X_i)\} + \dots$$

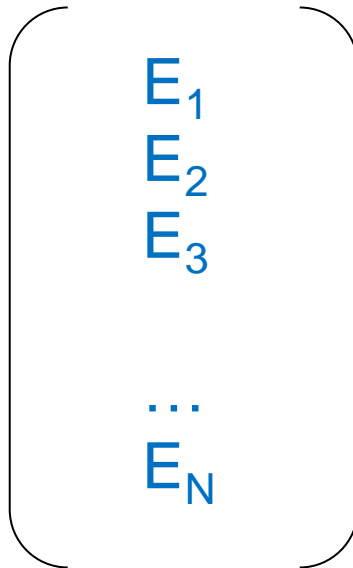
Unbiased Evidence-Based Approach

Evidence ($E_{1,2,3,}$) Supporting Triplet

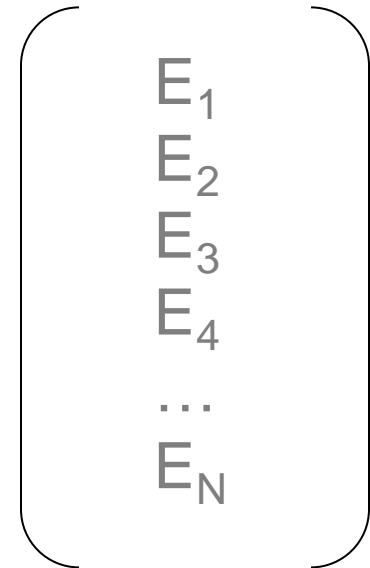
1. Scenarios



2. Likelihoods

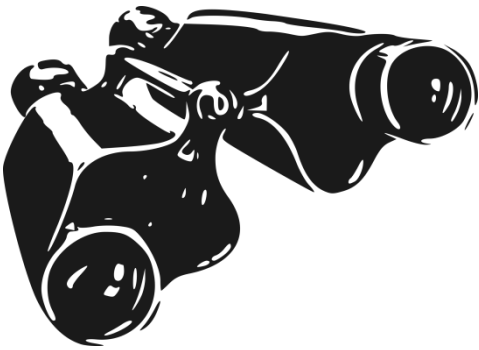


3. Consequences



Consider **all evidence**, with controls to **prevent selection of evidence** reflecting one belief (FDA) and excluding others

Focusing on Four Elements of Risk Assessment



1. Hazard Identification
- 2. Exposure Assessment**
- 3. Dose-Response Assessment**
4. Risk Characterization

1. Hazard Identification

What is important in causing or contributing to risks?

- Do **low exposures (small doses)** cause illness? (**infectivity**)
- Do standards or **safe levels** exist for pathogens of interest?
- How do people get sick (mechanism)?
- **How sick** do people get (severity, fatalities)? (**virulence**)
- Where are bacteria (environment, occupational exposures, ...)?
- How are people exposed (inhalation, ingestion, skin, ...)?
- How long are exposures that make people sick (acute, cumulative, ...)?
- What are predisposing and protective factors?



Hazard ID and Disease Triangle

Pathogens variable

- How **infective** for causing illness? (10 bacteria? A million bacteria?)
- How **virulent** for causing severe illness and fatalities?

Hosts variable

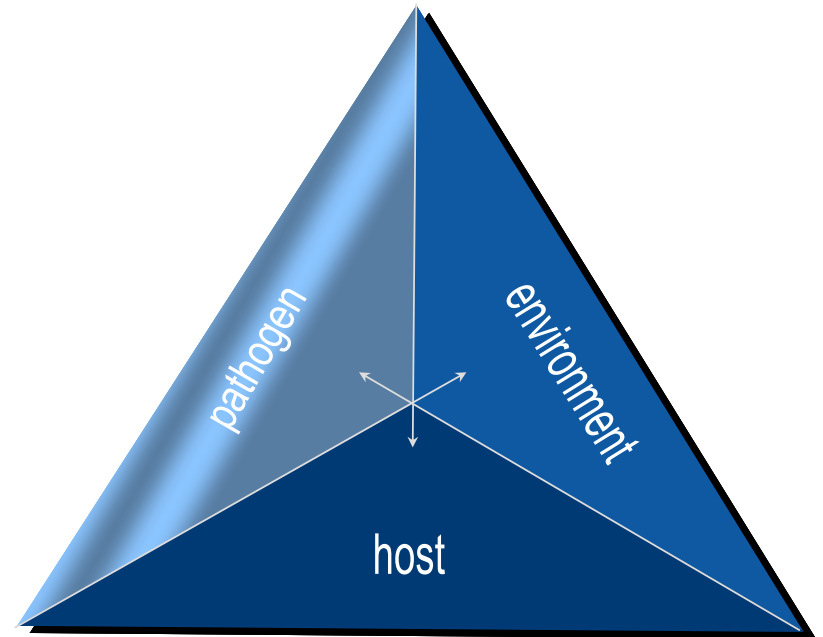
- Healthy (**resistant**) and more **susceptible** populations?

Environments variable

- Can affect pathogen infectivity and virulence, and host susceptibility and resistance.

Interactions complex!

- Seasonal, demographic, dietary, occupational, social and cultural, other factors **matter**.



Disease Triangle

2. Exposure Assessment

- Is a pathogen **detected** in milk?
- **How many** if detected?
 - Density (counts/mL or per serving) for positives
- Does pathogen **grow** (or die or survive) in milk?
 - If growth, **how fast** (or how long)?
 - Depends on **Temperature!**
- **How many** pathogens are in **simulated serving (DOSE ingested)** at consumption?



3. Dose-Response Assessment

Pathogen

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

Host

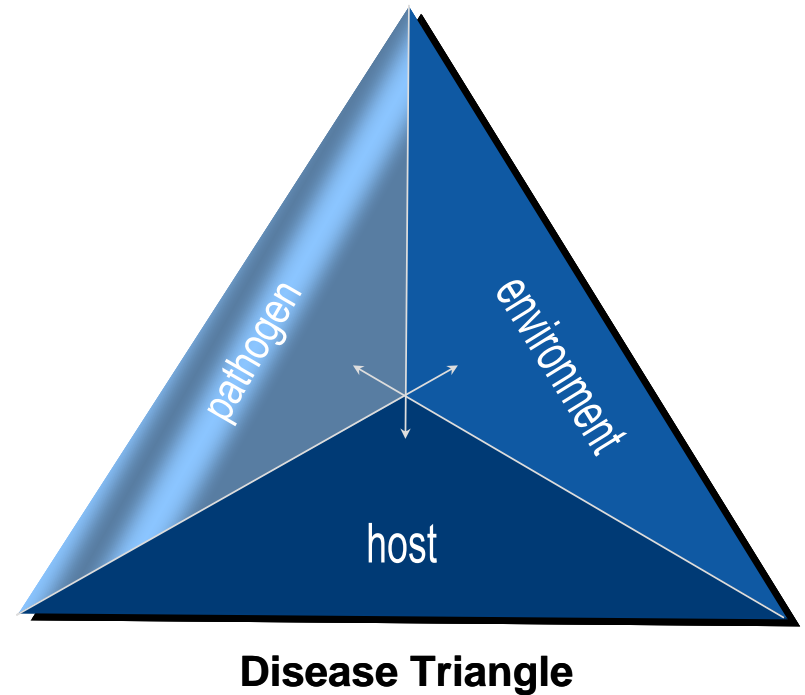
- Characterize dose-response relationships for populations at risk

Environment

- Characterize conditions causing disease

Interactions

- Characterize conditions favoring **sporadic disease** and **outbreaks**



Escherichia coli **NOT** a 'Superbug'

Enterohemorrhagic *E. coli* (EHEC, O157:H7)

- Caused outbreaks of **bloody diarrhea** with fatal complications
- Tragically, **4 children died** in US fast food outbreak from undercooked hamburgers (Jack in the Box) in 1993
- **Rare fatalities** in **recent decades** in US and worldwide
- **Key evidence:**

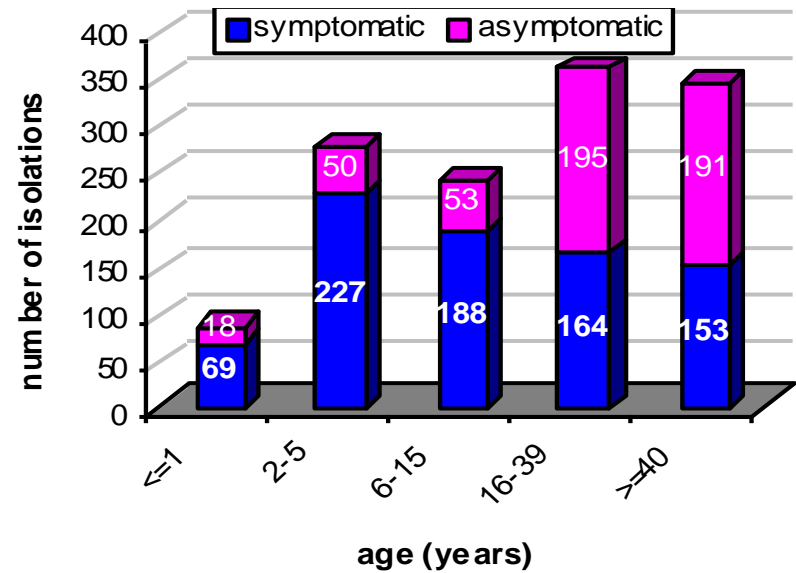
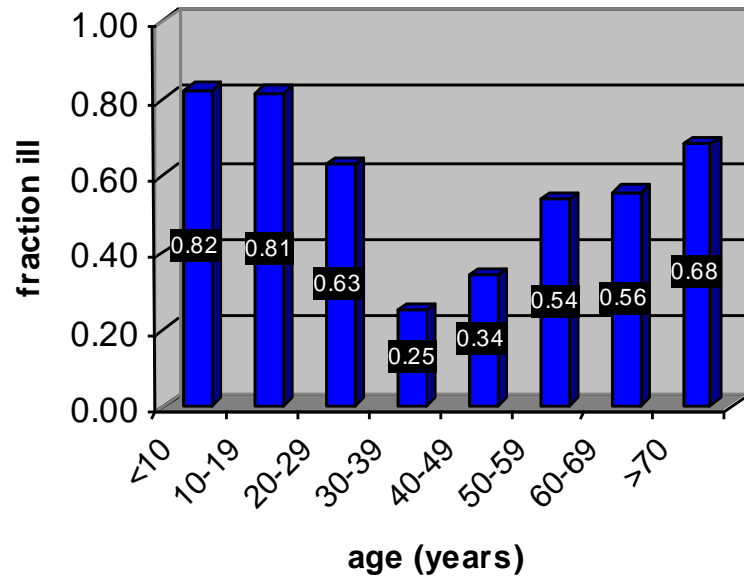


Japanese National Epidemiologic Surveillance,
Canadian farm family study, other sources



Many Exposed, Some Sick

(*E. coli* O157:H7 Positives)



(Wilson et al., 1996; Karmali et al., 1996; IASR, 1998; Haack et al., 2003)

4. Risk Characterization

How is it defined?

- Combine outputs of exposure assessment and dose-response assessment to estimate risk

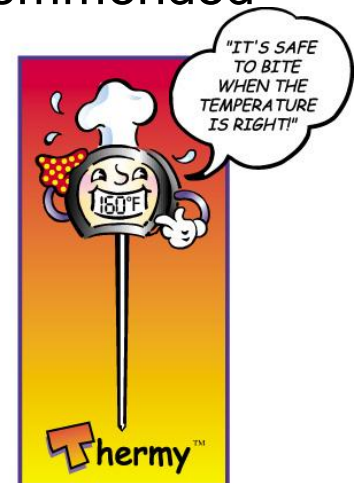
OR

- Compare output of exposure assessment to a 'safe' level derived from dose-response assessment to estimate increased risk above a threshold of negligible risk of illness
 - **Some 'safe' exposure guidelines selected, but controversial**
 - **10,000 *Vibrio* cells/g oyster**
 - **100 *Listeria monocytogenes*/gram food** recommended in Canada, European Union for foods not supporting growth; in US, regulators do not support this guideline

Risk characterization includes a description of uncertainty and its possible causes (sensitivity/uncertainty analyses)

Risk Communications: Revised as Science Advances

- Communication about safe cooking of **hamburgers** based on **visual cues** (brown not red color, texture, clear juices) were of questionable effectiveness in preventing illness.
- USDA study determined that these visual cues were **unreliable predictors of safety**.
- **NOW** use of thermometers or T-stick indicators is recommended to measure **temperature**, as well as practice proper food handling in the Fight Back Campaign:
 - Chill
 - Clean
 - Cook
 - Separate



Food Safety and Inspection Service, USDA

Risk Management: Cholera

Source: contaminated drinking water



Simple Solution in **London** (1854):

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



Simple Solutions Decrease Risk of Cholera

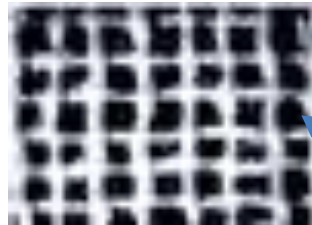
Source: contaminated drinking water



Simple Solution in **Bangladesh** (2000):

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

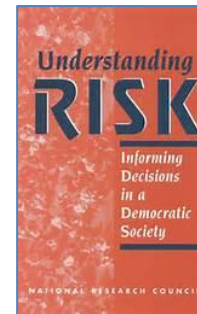
- Removed copepods that **concentrate** bacteria to **high doses**
- Scientific knowledge of **ecological link** of **copepods** and **cholera outbreaks** informed solution



cloth filters
copepods,
NOT *Vibrio*
bacteria

Risk Resources

- Many excellent resources (books, reviews, and technical papers) available in published literature, some **free full text**
- National Academy of Sciences, the National Academies Press (**free downloads** at <http://www.nap.edu/>)



- Journals (fees for online access without subscription)



Principles And Practice Exposure Assessment:

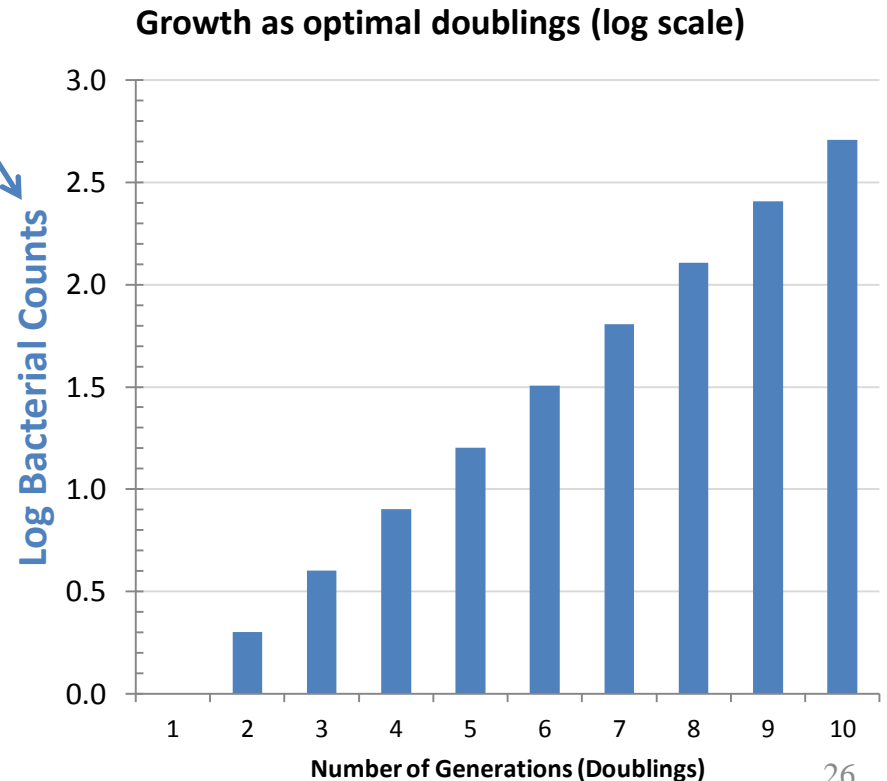
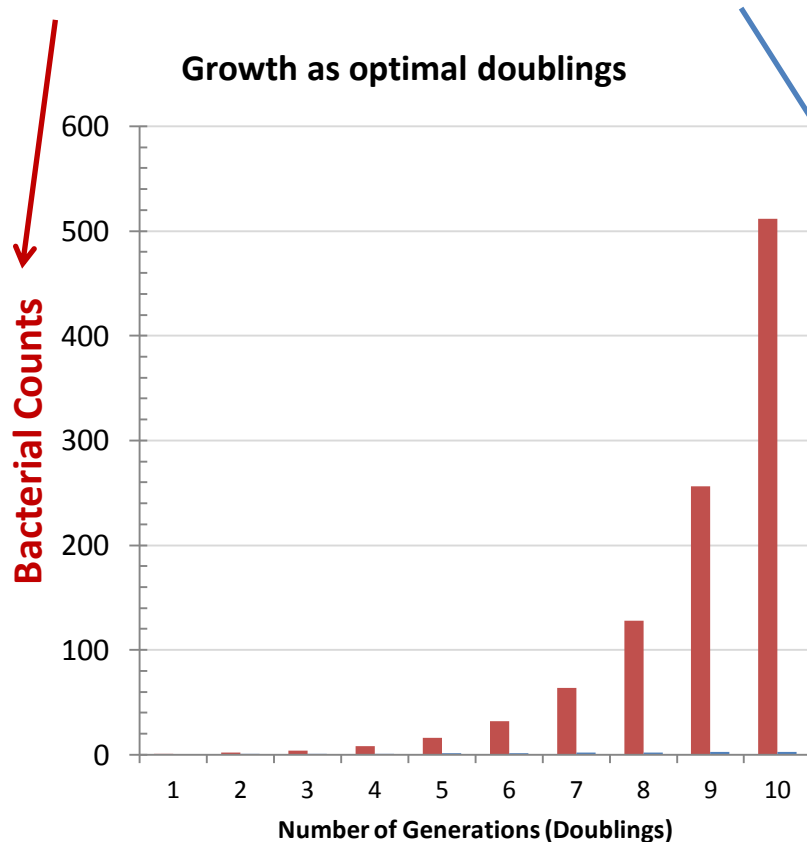
Examples from *E. coli* O157:H7 Experiments

Exposure Assessment and Bacterial Growth

Growth can be described as generations of **bacterial doublings**

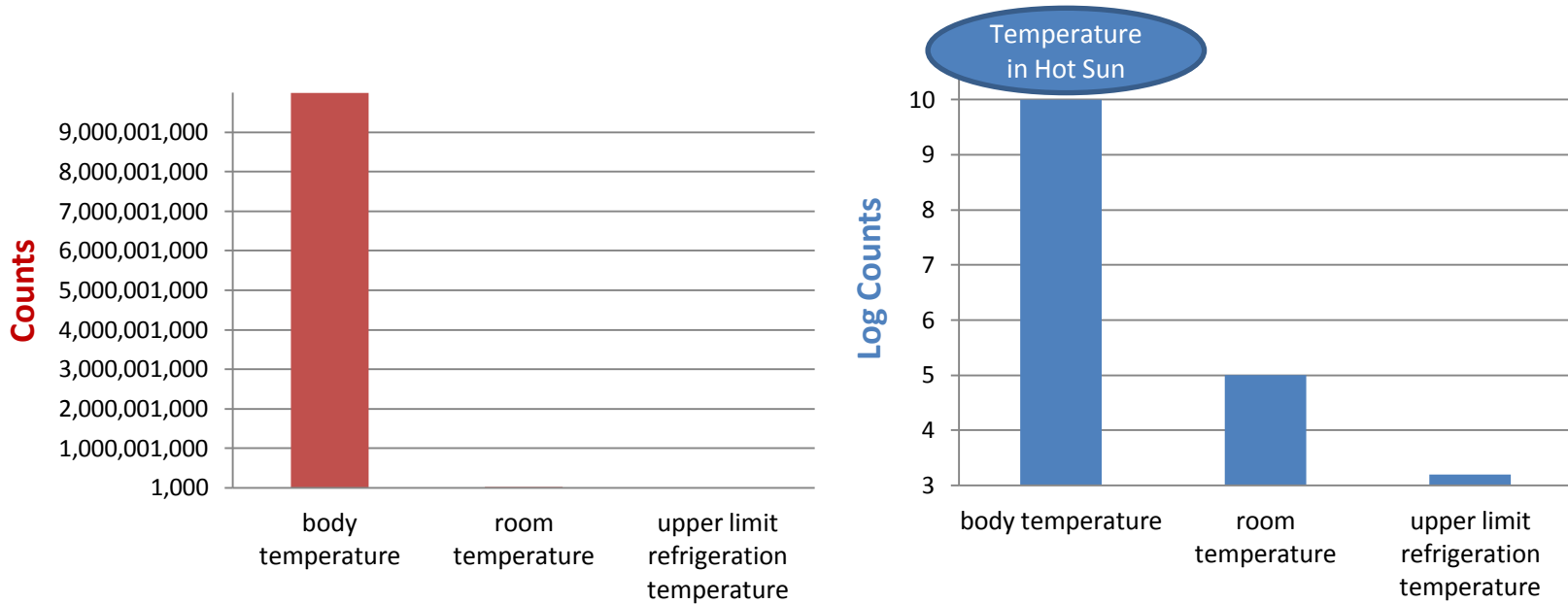
- One bacteria doubles, then those two bacteria both double, then those four all double, and so on
- For ten generations (cycles of doubling), same results are expressed as

COUNTS and **LOG COUNTS**



Why Use Log Counts?

E. coli growth in 12 hours across wide **temperature range** easier to compare



Count and Log Count Scales for Bacterial Growth

10 bacteria = 10^1 = log dose of 1

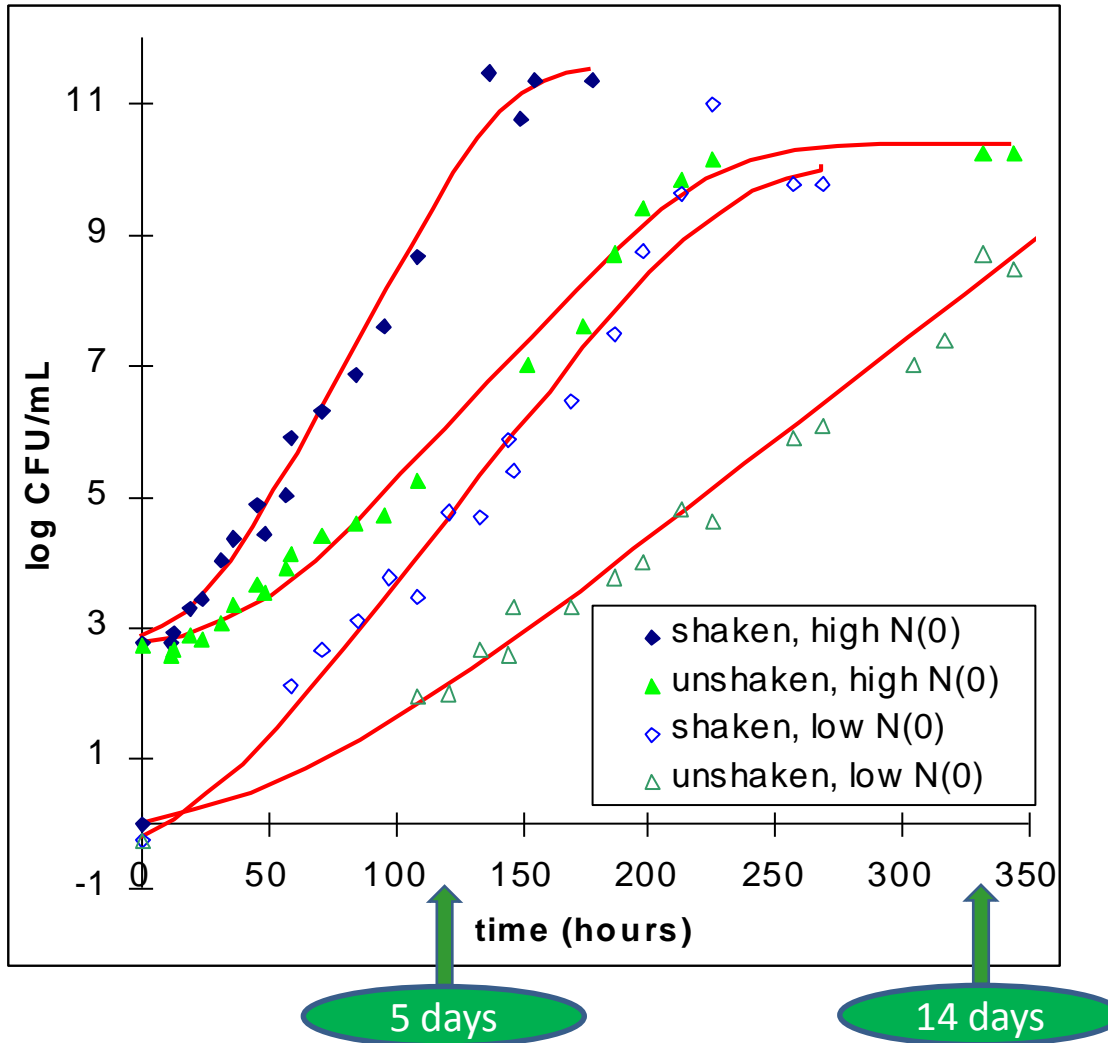
100 bacteria = 10^2 = log dose of 2

1,000 bacteria = 10^3 = log dose of 3

1,000,000 or a million bacteria = 10^6 = log dose of 6

10,000,000,000 or ten billion bacteria = 10^{10} = log dose of 10

E. coli O157:H7 Growth at Sub-optimal Conditions



- **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**

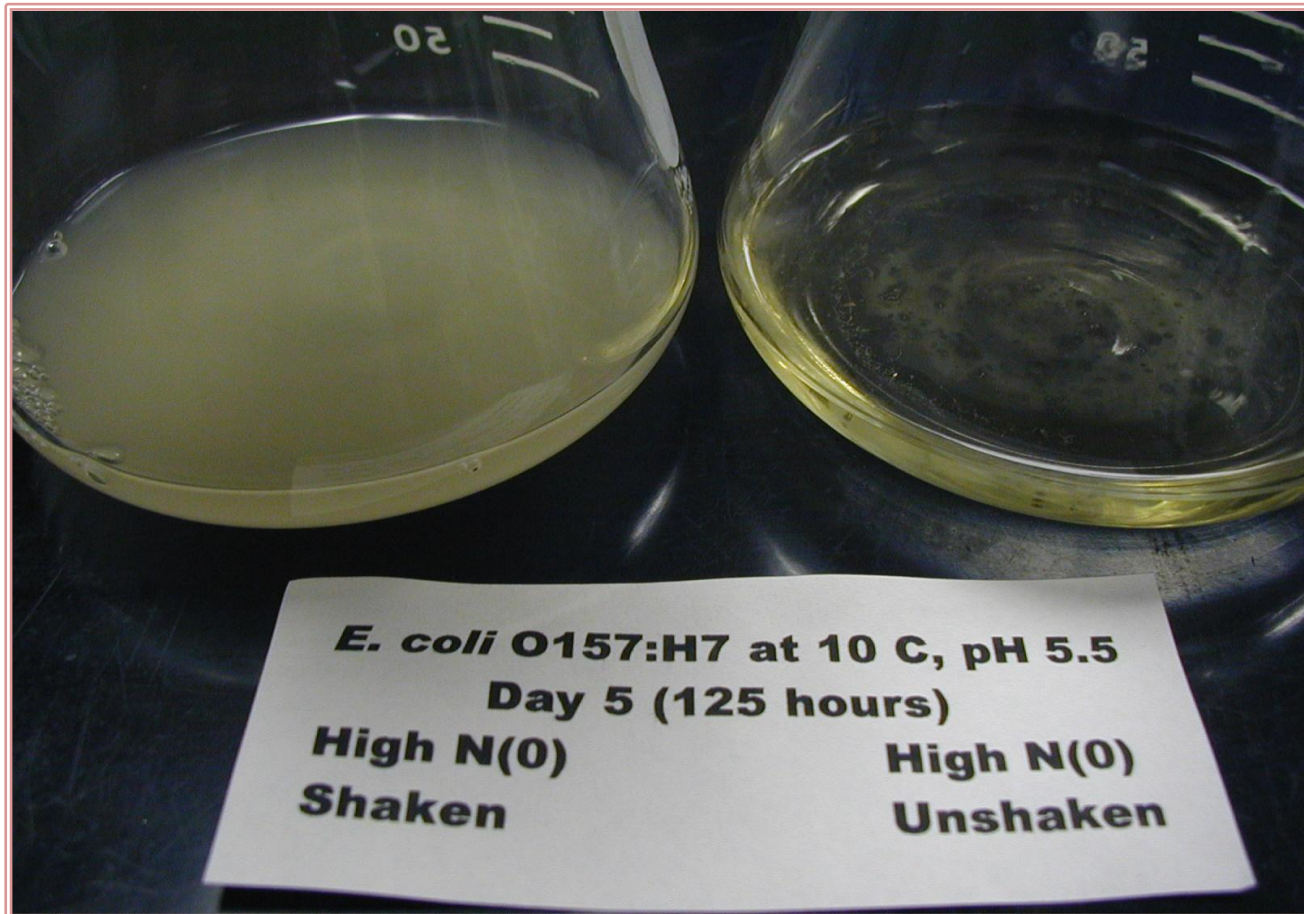
(like milk bottle in refrigerator versus culture flask on rotating shaker 24-7)

(Coleman et al., 2003)

Sub-optimal Conditions Limit Pathogen Growth

Shaken Flasks, **Visible** Growth (cloudy), Unshaken Flasks, **No Visible** Growth (clear)
after **5 days** refrigeration

Optimal
(with shaking)



Sub-Optimal
(no shaking)

Beyond Laboratory Flasks: **Microbiota**

A dense ecological community of **commensal**, **symbiotic** and potentially **pathogenic** microbes that literally share our body space

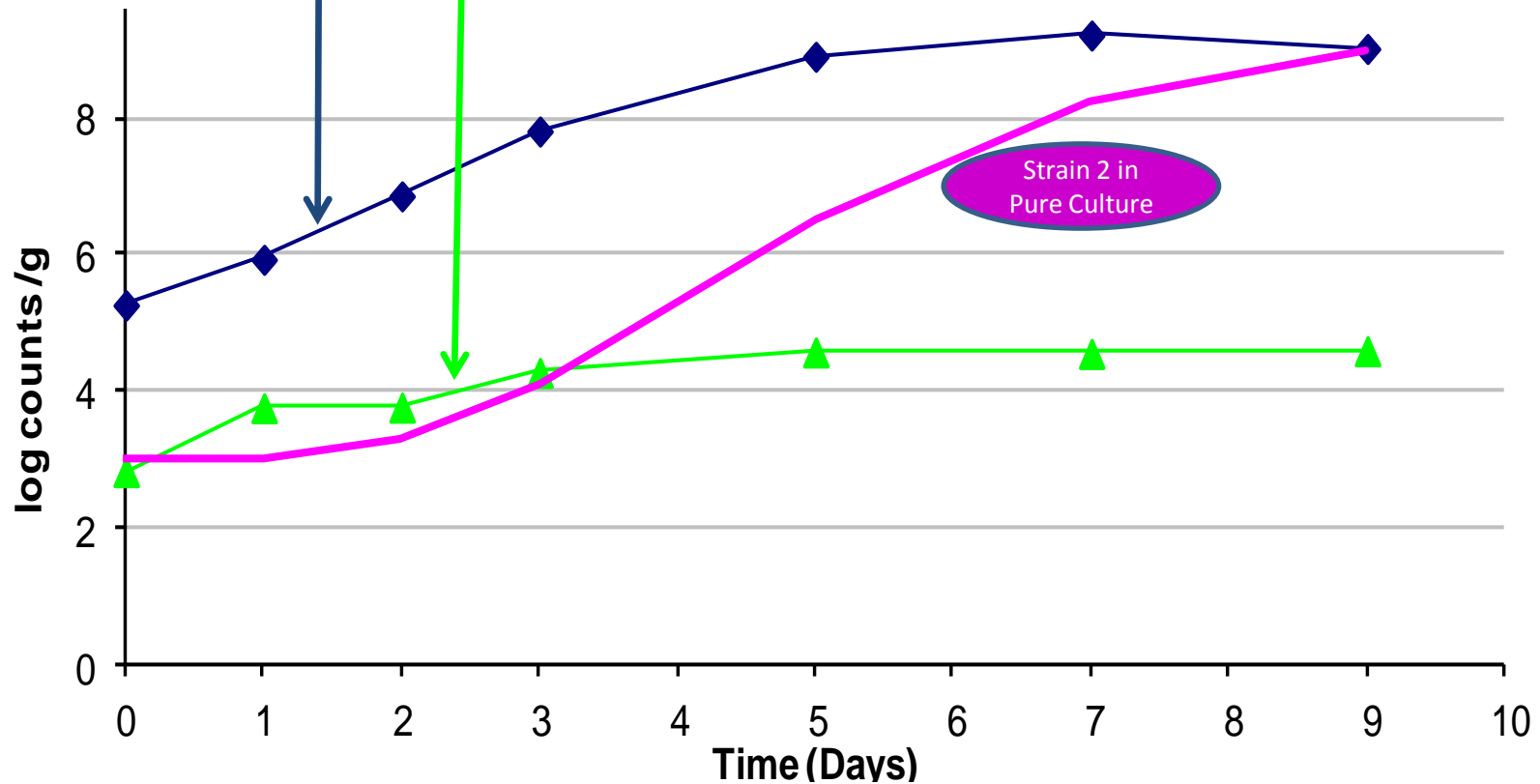
Commensal - relationship between two organisms where one organism gets food or other benefit from the other organism without helping or hurting it.

Symbiotic - relationship between two different organisms where there is benefit for both of them.

Pathogenic - relationship between two different organisms where one is capable of causing disease in the other.

Microbiota of Ground Beef Inhibits

E. coli O157:H7 (Strain 2)

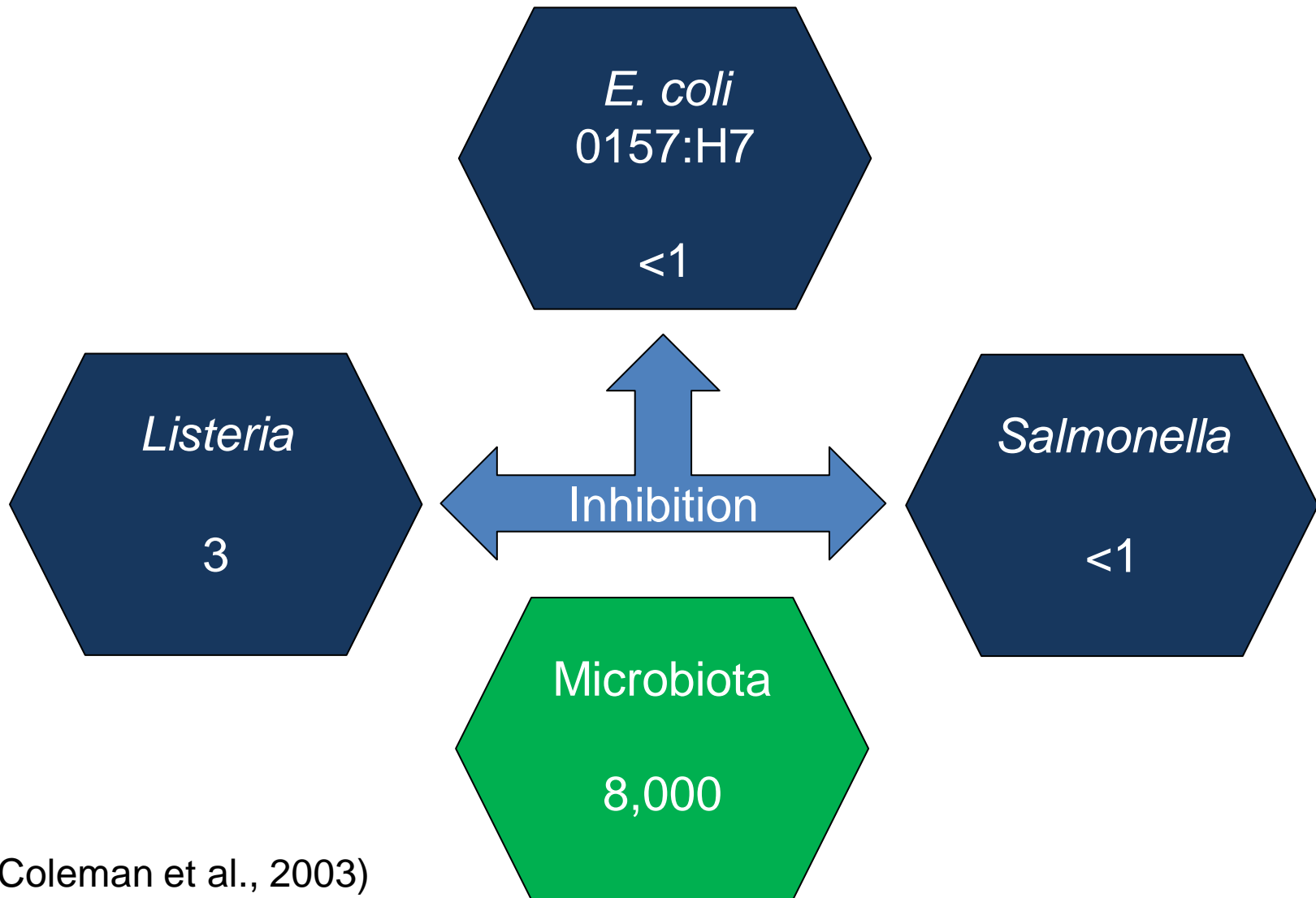


Ground beef **microbiota** measured by **total plate count**, predominated at refrigeration temperatures by non-pathogenic *Pseudomonas* spp., inhibits **Strain 2** growth. Optimal pathogen growth in pure culture flasks in **pink** (Pathogen Modeling Program).

(Tamplin, 2001)

Numerical Dominance of Ground Beef Microbiota

(counts/gram)



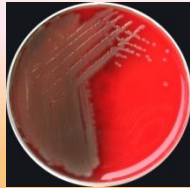
(Coleman et al., 2003)

Another Ecological Advantage:

Microbiota Grows **Faster** than **Pathogen**



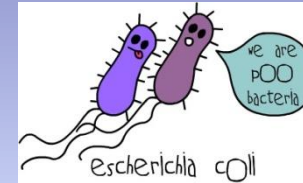
Pseudomonas



36° F	0.09/hr
39° F	0.11/hr
50° F	0.24/hr



Escherichia coli O157:H7



36° F	no growth
39° F	no growth
50° F	0.03/hr

Pseudomonads grow at the lowest temperature,
while pathogen does not grow at all

Exposure Assessment Issues for Foods

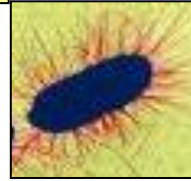
- **Optimal growth conditions** in laboratory experiments **unrealistic** for non-sterile foods (sub-optimal growth)
- Microbial growth depends on
 - How many **pathogens** present in foods (typically 1, 10, or <100, **not thousands or more**)
 - How many **competing microbes** present in foods (**tens of thousands or more in microbiota of 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 **microbiota**

Principles And Practice Dose-Response Assessment

Examples for Anthrax, Tularemia, Salmonellosis, Campylobacteriosis

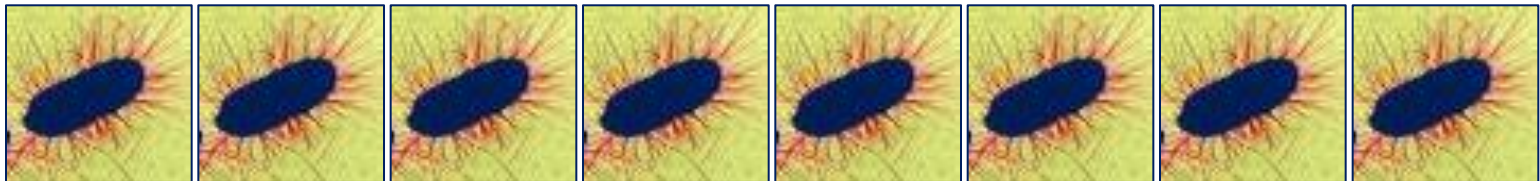
Dose: exposure to one pathogen



Response: illness unlikely

Dose-Response Relationships

Dose: exposure to many pathogens



Response: illness as dose increases

Dose: exposure to 2,000 pathogens

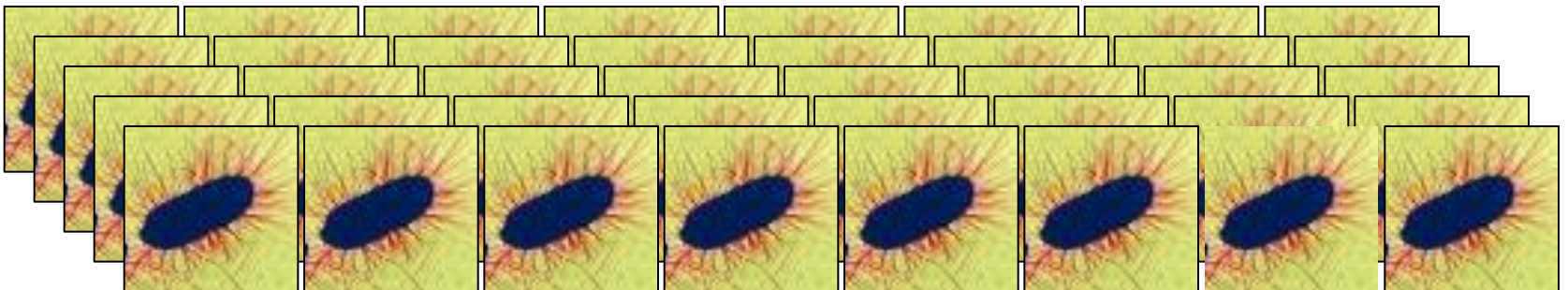
Guess the Pathogen!



Response: no adverse effects

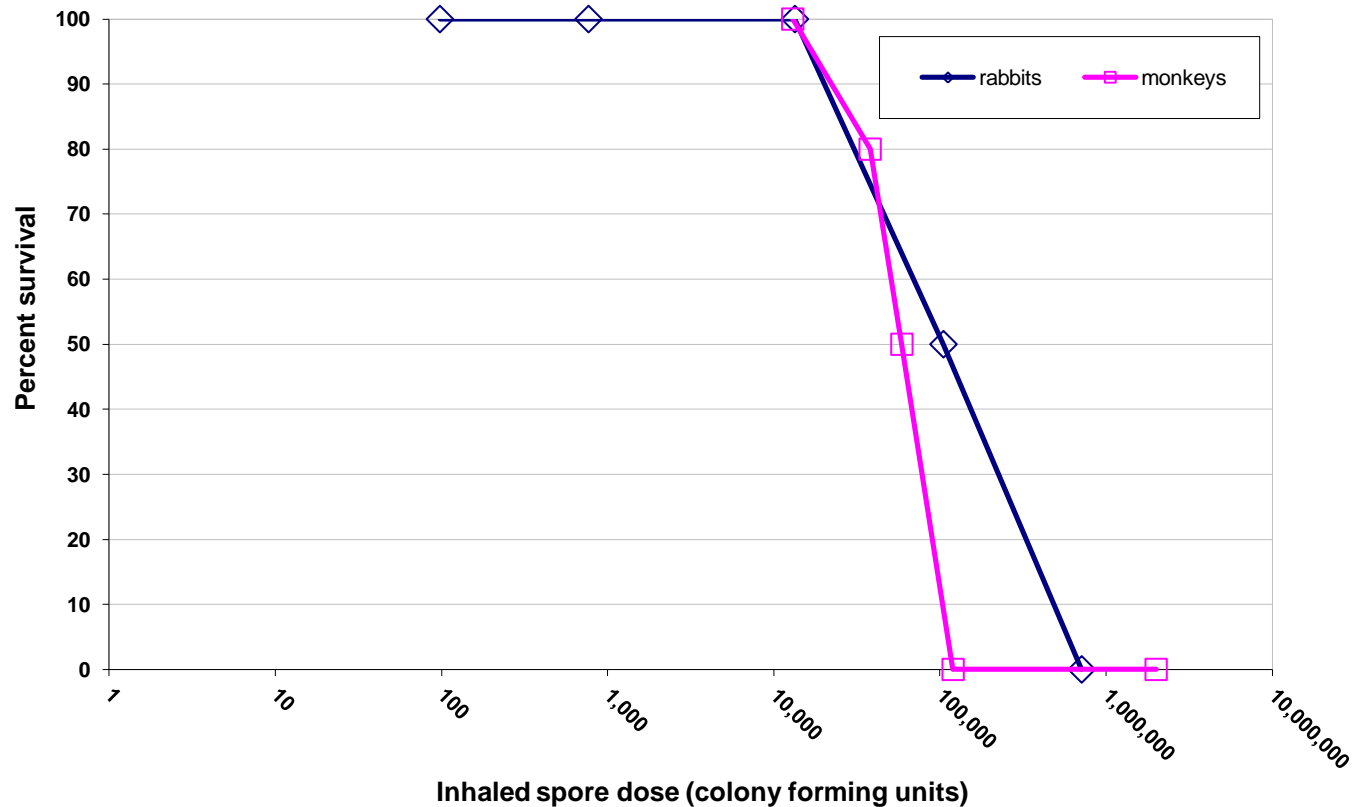
Actual Dose-Response Relationship

Dose: exposure to >100,000 pathogens



Response: illness observed

Probability of Surviving Anthrax Exposures

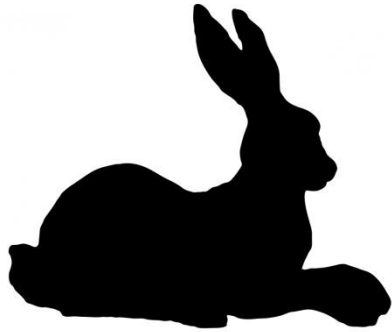


100% survival (zero mortality)

for doses of 100, 1,000,
and 13,000 spores

(Pitt, 2004)

Relating Animal Data to Human Dose-Response



What can we learn from animals?

Risk for Mice and Men

(and Women, Children, Elderly)

Animal models **not all representative of human responses** (infectivity, virulence, pathology and defenses, particularly immunity)

Listeriosis

- Mice don't develop gastrointestinal (GI) symptoms because the pathogen doesn't adhere in the gut (**no receptor**)
- Humans, non-human primates, guinea pigs, ferrets all develop GI symptoms (**have receptor**)



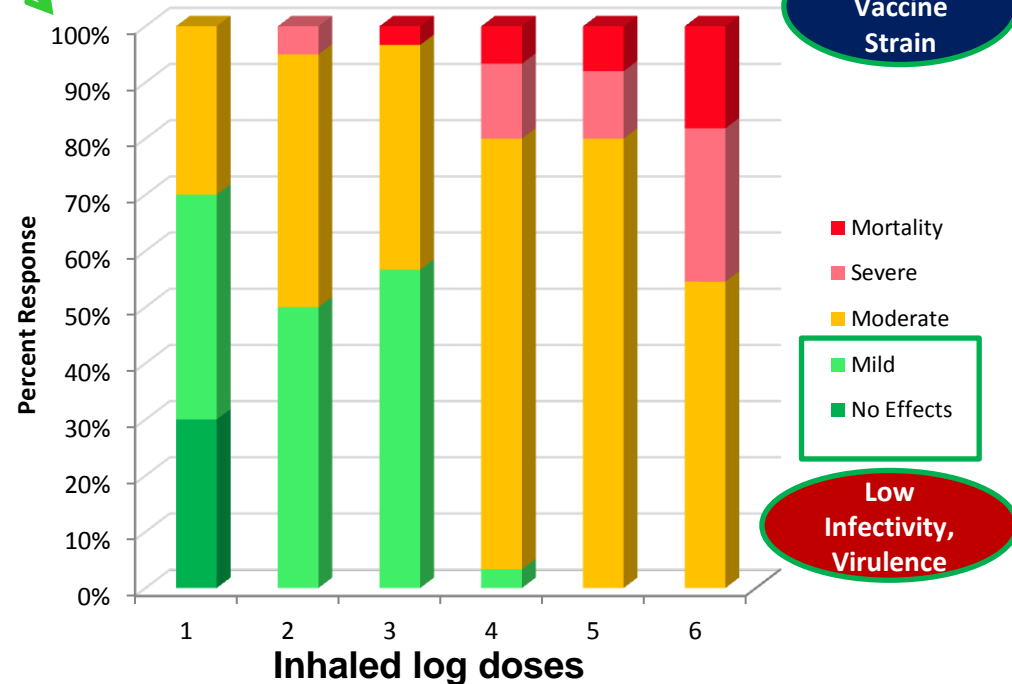
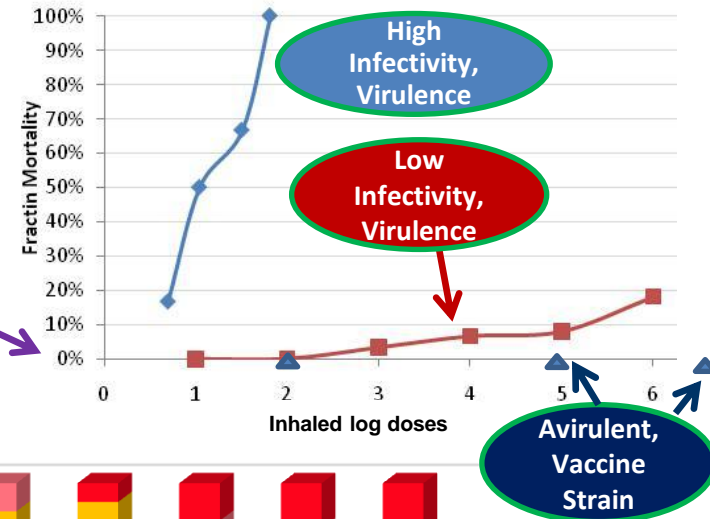
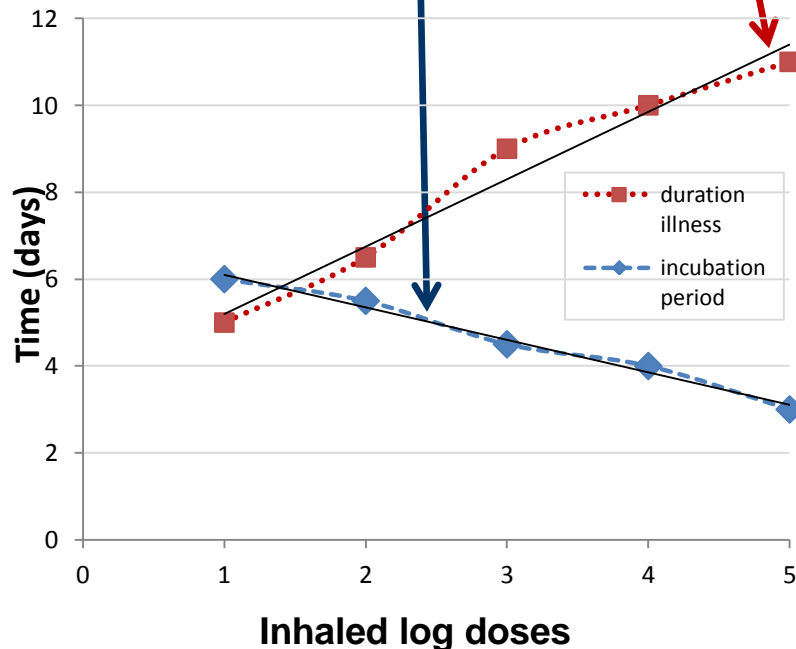
Tularemia

- Primates react differently to strain types than rabbits and rodents. Even a vaccine strain that causes no illness in humans will kill mice because their immune system is different.

Dose-Responses for Tularemia in Monkeys

Increasing pathogen dose

- Increases **likelihood of illness** by strain
- Increases **severity of illness**
- Increases **duration of illness**
- Decreases **incubation period** before onset of symptoms



Evidence for Thresholds for Human Illness

Healthy people can **tolerate low doses** of many pathogens. Volunteers got sick at **high doses** for some strains in these human clinical studies.

Salmonellosis cases observed at doses greater than 10^9 or **1,000,000,000** ingested bacteria for one of six serotypes (pullorum)

(McCullough and Eisele, 1951;
Coleman and Marks, 2000)

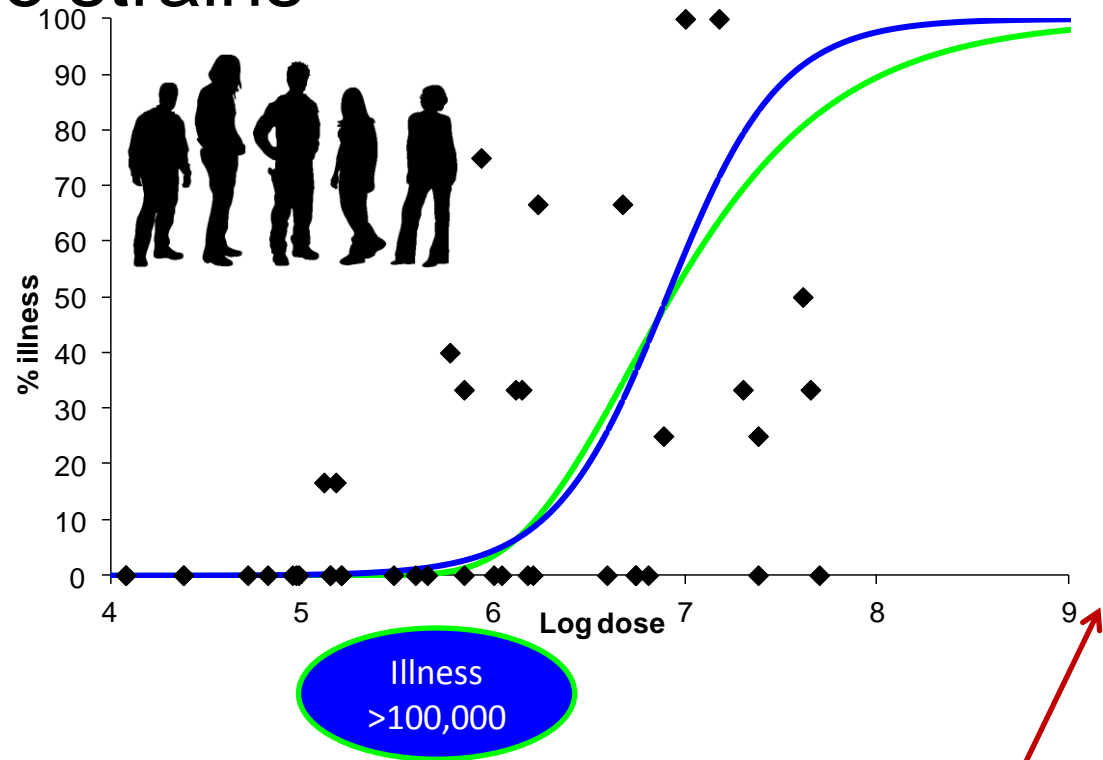
Tularemia cases observed at doses greater than 10^6 or **1 million** ingested bacteria (*Francisella tularensis*)

(Hornick et al., 1966; B. Thran, 2015)

Salmonella Strains Administered to Humans

Modeling multiple strains

- anatum
- bareilly
- derby
- meleagridis
- newport



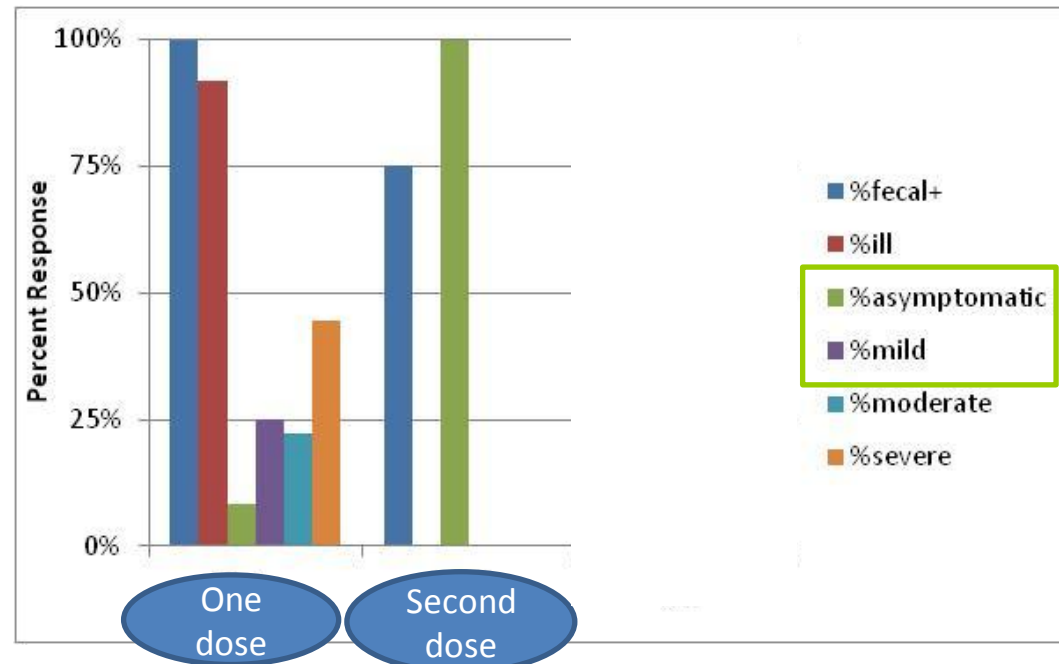
- **pullorum (statistically significant threshold at 10⁹ bacteria)**

(Coleman and Marks, 1998;
McCullough & Eisele, 1951a-d)

Campylobacter Administered to Soldiers

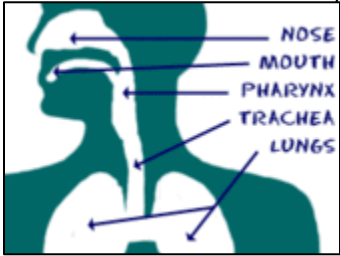
Factors Influencing Travelers Diarrhea

- **Innate immunity** in volunteers, even after **one high dose** (**10⁹** or **one billion bacteria**)
- **Adaptive immunity** (antibodies) protect after **second dose** over time, all asymptomatic in short term
- **Previous antibiotic** treatment
- Fatigue, physical and psychological stress
- Boredom with ready-to-eat meals
- Failure of **public health advice** to prevent **travelers' diarrhea**



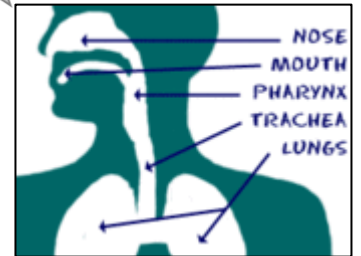
Avoid street vendor foods/beverages, raw and undercooked meat/seafood, raw fruits/vegetables, tap water, ice, **unpasteurized dairy products**

500 spores



survival

?10,000 spores?



mortality

Human Resistance to ANTHRAX !?!?!

Uncertain where to draw the line for 'safe doses'

STRETCH BREAK!

Feel free to stand up and
introduce yourself to someone
near you!

Risk Perceptions And Estimates For Listeriosis For Milk

Listeriosis Predictions for Milk

Perception: FDA Division Plant & Dairy Food Safety (Sheehan,2012)

- Raw milk is **poison**, consuming is **playing Russian roulette** with your health

Estimates: FDA Risk Assessment Division (2003), with USDA

- US consumers **exposed regularly** to **low to moderate levels** of *Listeria monocytogenes* in foods (23 categories investigated)
- Exposure and Dose-Response models **overpredict** risk

Pasteurized Milk

- 90.8 deaths per year (**high** risk)
- 10^{-9} per serving or 1 fatal case in 1,000,000,000 exposures at selected doses (**moderate** risk)

Unpasteurized Milk

- 3.1 deaths per year (**moderate** risk)
- 7×10^{-9} per serving or 7 fatal cases in 1,000,000,000 exposures at selected doses (**high** risk)

Fatal cases predicted, but not observed in decades!

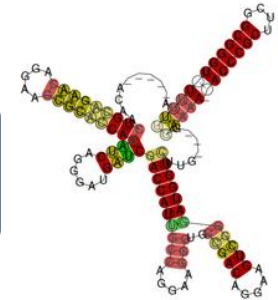
FDA Exposure Assessment

FDA (2003) Bias

Predicts simple exponential growth, not accounting for milk (or gut) **microbiota**



OVERPREDICTION of *Listeria* Growth



Dense milk microbiota (>10,000 bacteria per mL) **outgrows**
low levels of pathogens

Milk microbiota predominated by faster growing **non-pathogens**,
typically **pseudomonads**

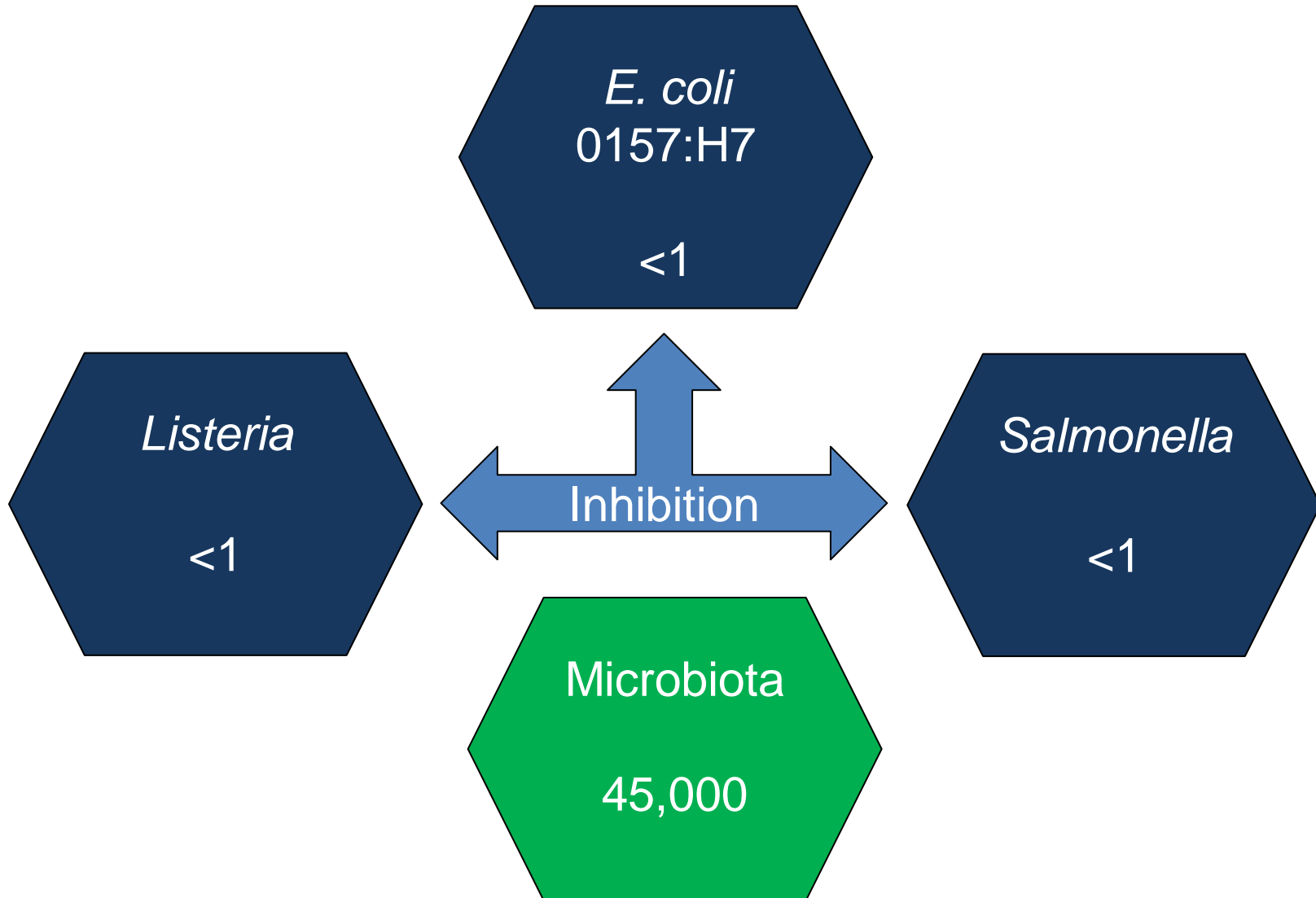
Raw Milk Microbiota Out-Competes Pathogens

Dairy Study	Numbers of Raw Milk Positives (range; mean; median) in CFU/mL				
	Standard Plate Count	<i>Listeria monocytogenes</i>	STEC/VTEC	<i>Salmonella</i> spp.	<i>Staphylococcus aureus</i>
D'Amico et al., 2008 Farmsted dairies N=62	62	3	0	0	17
	(10 to 10 ⁵ ; 4.9x10⁴ ; 7.0 x10 ²)	<1	Non-detectable	Non-detectable	Unspecified; 250; <1
	Total Viable Count	<i>Listeria monocytogenes</i>	STEC/VTEC	<i>Salmonella</i> spp.	<i>Bacillus cereus</i>
Jackson et al., 2012 Commercial dairy silos N=184	184	23	30	5 - 33	4
	7x10 ² to 5x10 ⁵ 4.2 x10⁴	<0.006 to 29	<0.006 to 1.1	<0.006 to 60	3 to 93
	-	0.65 0.12	0.19 0.26	0.75 0.12	0.75 0.12

Temperature (°F)	<i>Pseudomonas</i> spp.	<i>Listeria monocytogenes</i>	<i>Escherichia coli</i> O157:H7	<i>Salmonella</i> spp.	<i>Campylobacter</i> 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 (Coleman et al., 2003)

Numerical Dominance of Raw Milk Microbiota (counts/mL)

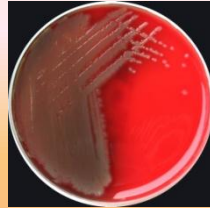


Another Ecological Advantage:

Microbiota Grows **Faster** than **Pathogen**

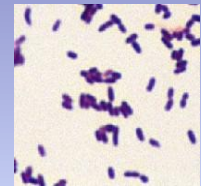


Pseudomonas



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
while pathogen does not grow at all

Research for Exposure Assessment



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** if **≥ 100 CFU/mL *Listeria*** is detected

- Evidence for growth/**no growth** of pathogens in raw milk?
If no growth for *Listeria*, **raw milk at <100 CFU/mL** could be considered **unadulterated** (acceptable or tolerable or 'safe')
- **Milk microbiota studies** could better characterize microbes associated with suppressive effects on *Listeria* and other pathogens

FDA Dose-Response Assessment

Dose-Response Model for Listeriosis in Milk

- Mouse mortality model **not representative** of human listeriosis
 - needed to shift dose-response curve more than **10 orders of magnitude** in order to make fatality predictions consistent with human epidemiologic data
- **No scientific evidence** support the shift or explains discrepancies between hosts:
 - low morbidity (illness) and high mortality (death) in mouse;
 - low morbidity and low mortality in humans
- Recent data on **protective effects** of GI microbiota against *Listeria* not considered

(Archambaud et al., 2012; Gomez et al., 2012; Nakamura et al., 2012)

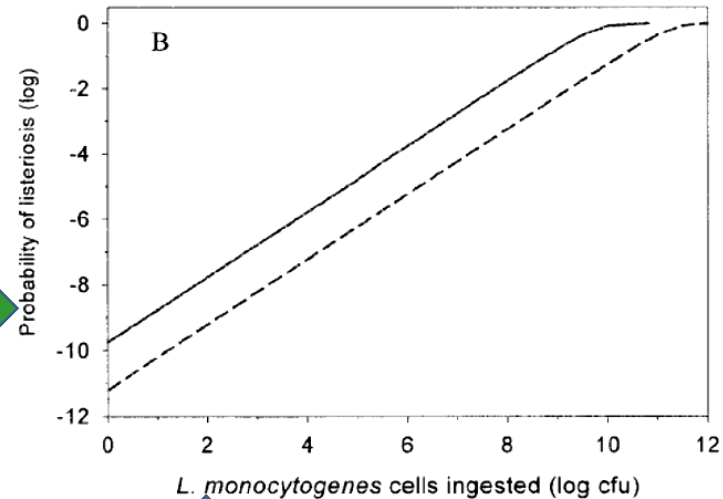
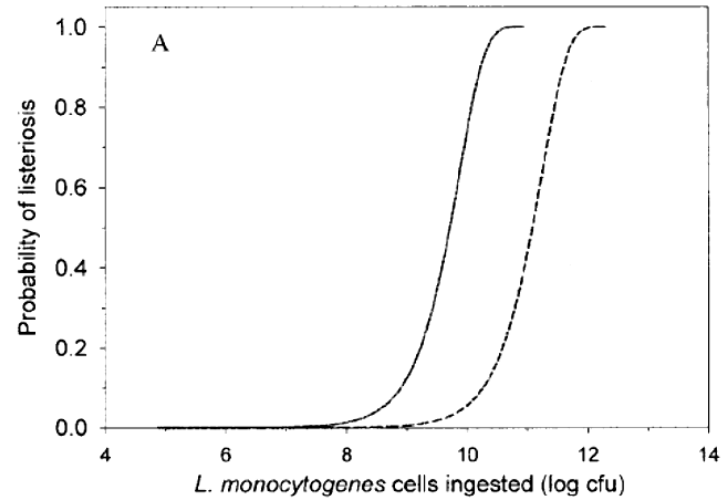
Overprediction of human GI cases due to use of **mouse mortality** model

Risk Characterization Using Alternative Dose-Response Model A

L. MONOCYTOGENES **LOW LEVELS EQUAL LOW RISK** 573

Consider exposure for raw milk of **100 counts** (1 count/mL for 100 mL serving) **without growth or decline**

Likelihood of GI illness from Chen's human DR model is **1 illness in 1,000,000,000 servings (10^{-9})** or **1 in a BILLION servings!**
NOT HIGH RISK !!!



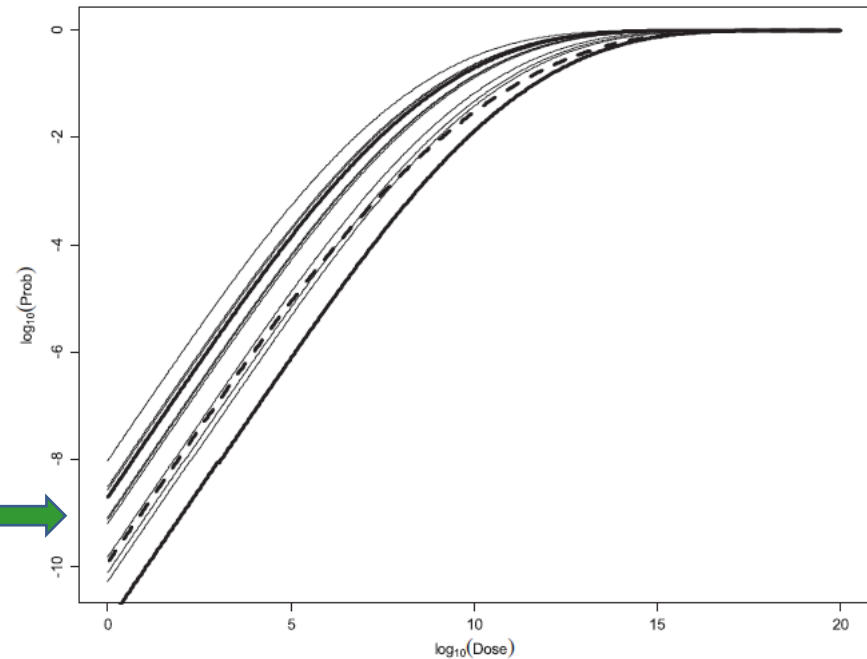
(Chen et al., 2003)

Risk Characterization Using Alternative Dose-Response Model B

Consider exposure for raw milk of **100 counts** (1 count/mL for 100 mL serving) **without growth or decline**

DR modeling updated in 2015 suggests that most listeriosis cases are linked to the ingestion of food contaminated **with >10,000 *L. monocytogenes* !**

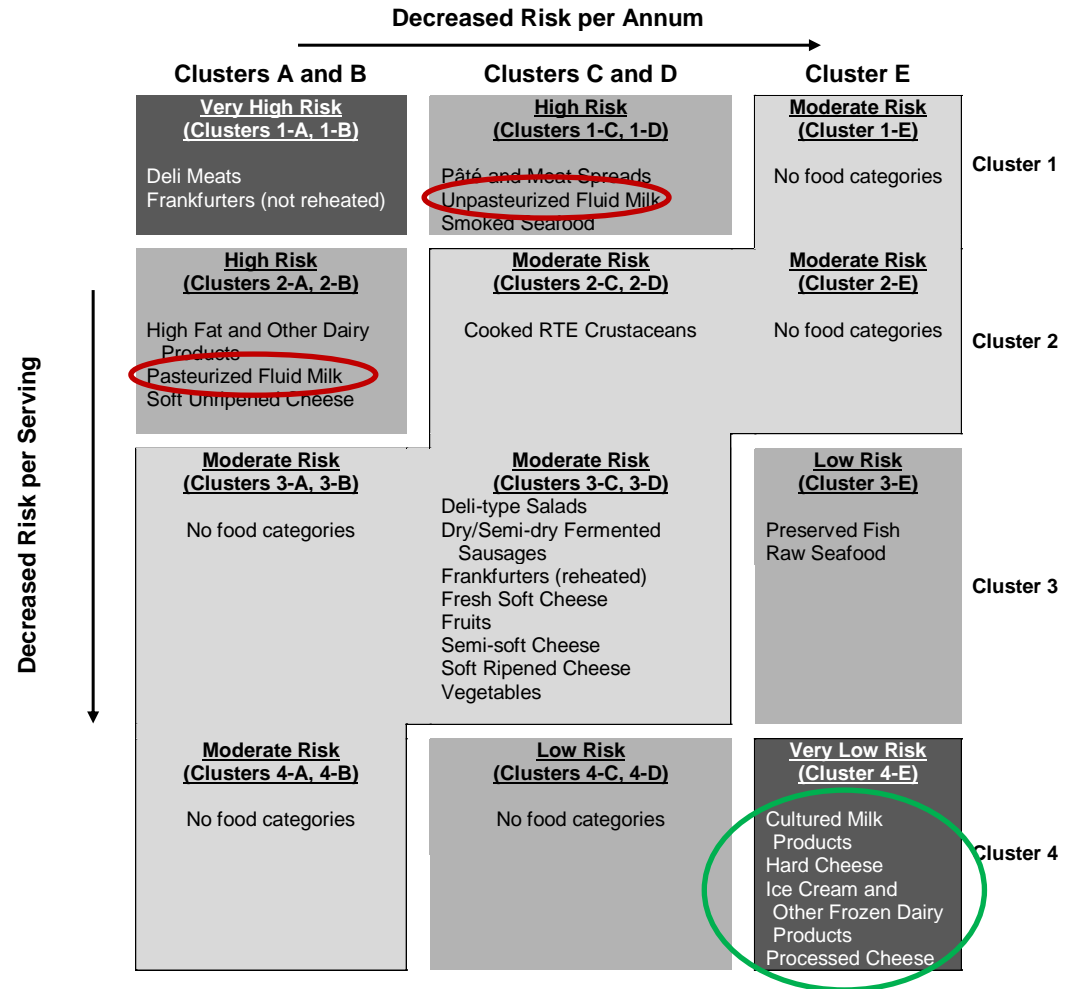
Likelihood of GI illness from Pouillot's human DR model is **1 illness in 1,000,000,000 servings (10^{-9}) or 1 in a BILLION servings!**
NOT HIGH RISK !!!



(Pouillot et al., 2015)

Validation for Relative Risks of Listeriosis?

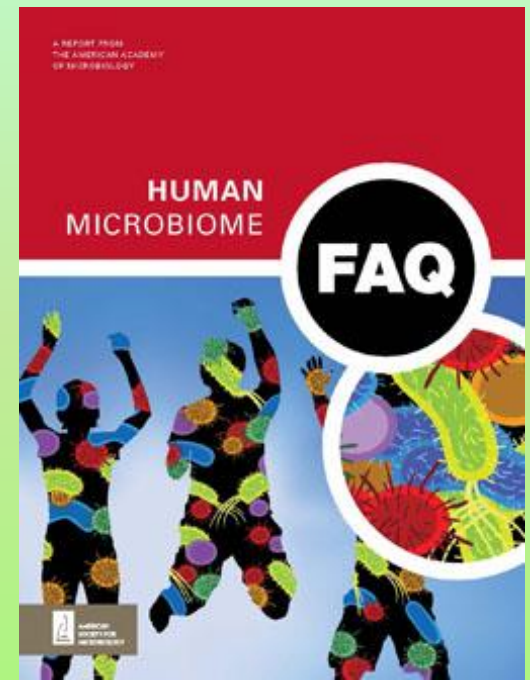
- **Very high and high risk foods** (upper left) **alleged** to support growth, including **pasteurized** and **unpasteurized milk**
- **Very low and low risk foods** (lower right), including frozen foods and hard cheeses, **allegedly have intrinsic or extrinsic factors to prevent the growth** or are processed to alter the normal characteristics of the food



Research Needed for Reliable Human Dose-Response Assessment

- **GI microbiota studies** may demonstrate the presence of higher levels of protective microbes in consumers of raw versus pasteurized milk
- **Studies of dairy farm families and workers** may demonstrate greater exposure and greater immunity to *Listeria*, *Campylobacter*, and other pathogens in consumers of raw versus pasteurized milk, as protective microbiota of workers handling chickens confers resistance to campylobacteriosis





Natural Microbiota Protects Against Pathogens

Human Microbiome

What is it?

- Community of trillions of bacteria and other microbes living in intimate association with our bodies, and the genes they contain

What are they doing?

- Helps us extract energy and nutrients from food
- Crowds out or **inhibits pathogens** (competitive exclusion)
- Enhances and **'orchestrates' immune responses** and gut barrier function
- **Maintains balance** (homeostatis)
- Alters intestinal mobility or function

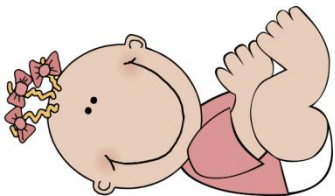


(<http://academy.asm.org/index.php/faq-series/5122-humanmicrobiome>)

How Do We Get Our Microbiome?

Birth

A newborn gets its microbes from its mother's birth canal, skin of its **mother** and other **caregivers**



Unpasteurized Breast Milk

Breast milk has been fine-tuned over millions of years to provide nutrients, vitamins, antibodies, **diverse microbes** to populate the baby's gut



Environment

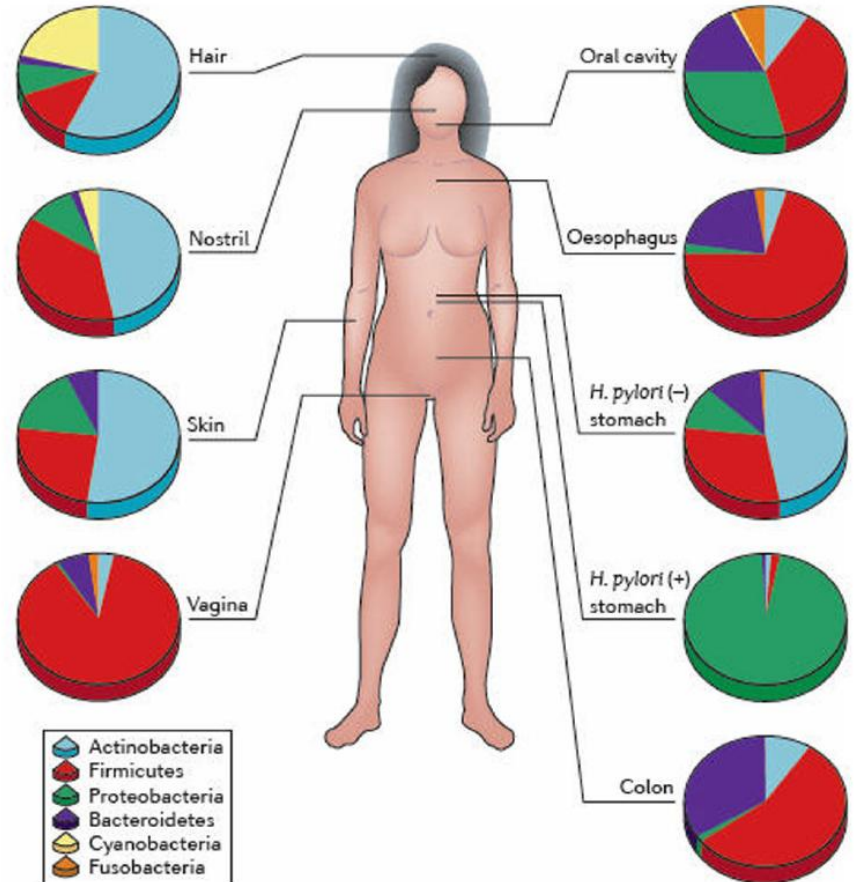
We continuously encounter bacteria everywhere, from air, water, people, pets, soil, plants, and **foods** daily



(<http://academy.asm.org/index.php/faq-series/5122-humanmicrobiome>)

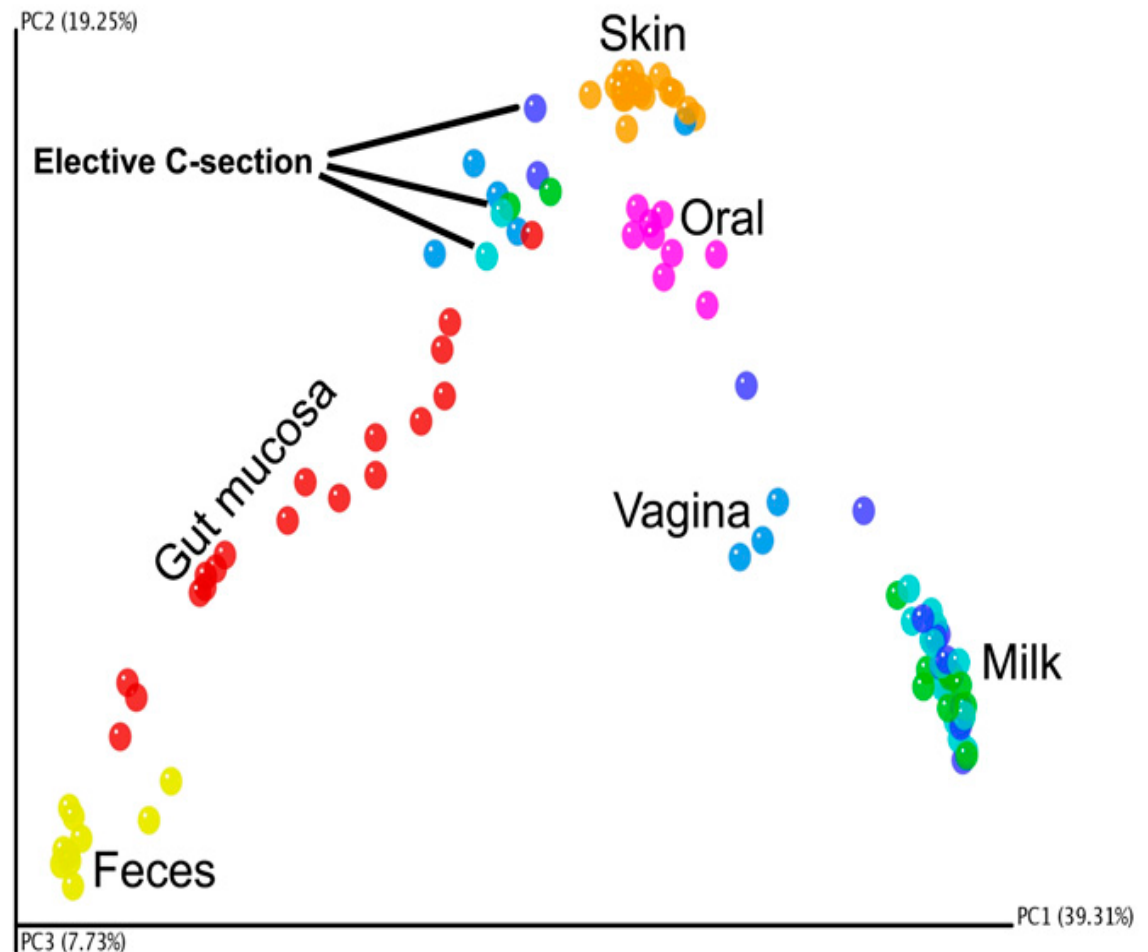
Healthy Human Microbiomes

- **Human Microbiome Project** (2007-present)
- Results **continue to challenge old dogma:**
 - Many healthy human tissues were **presumed sterile** (free of microorganisms)



Relatedness of Human Microbiota

- **Not random associations**, some niches with 'core' groups present or dominant in most individuals
- **Gut microbiota** are more similar between individuals than to skin or oral or other microbiota of same individual
- Stability and resilience high, but can be disturbed.



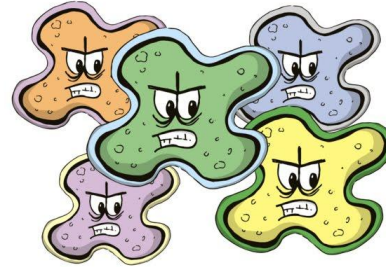
GI Microbiome in Health

- **Food-Borne Microbes:** Shaping the **Host** Ecosystem
 - Estimate of **10^{10} (10,000,000,000 or TEN BILLION!)** microorganisms as **daily dietary consumption**, most commensals and few pathogens (<0.1% abundance)
 - Most pass through the intestines into feces without attaching to (or infecting) any human cells
- Findings of **NIH Human Microbiome Project**
<http://commonfund.nih.gov/hmp/>
 - GI microbiome includes diverse consortia of **>40,000** microorganisms
 - **10^{14} (100,000,000,000,000!)** microorganisms typically present in human colon
 - **Complex** spatial and temporal gradients
 - **NOT** simple well-mixed flasks of nutrient media



(Jaykus et al., 2009)

Dysbiosis

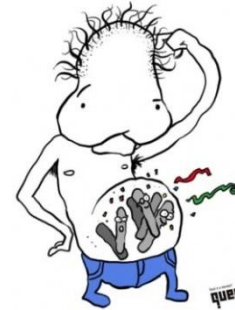


- Refers to **microbial imbalance** resulting from a change in the number or types of bacteria on or inside the body.
- Is most prominent in the **digestive tract** or on the **skin**, but can also occur on any exposed surface or mucous membrane.
- May have a role in illnesses such as inflammatory bowel 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)

Colonization Resistance

- Microbiota of healthy people can effectively **inhibit colonization** and **overgrowth** by invading pathogens. This phenomenon was observed in 1954 and termed **colonization resistance** in 1971.



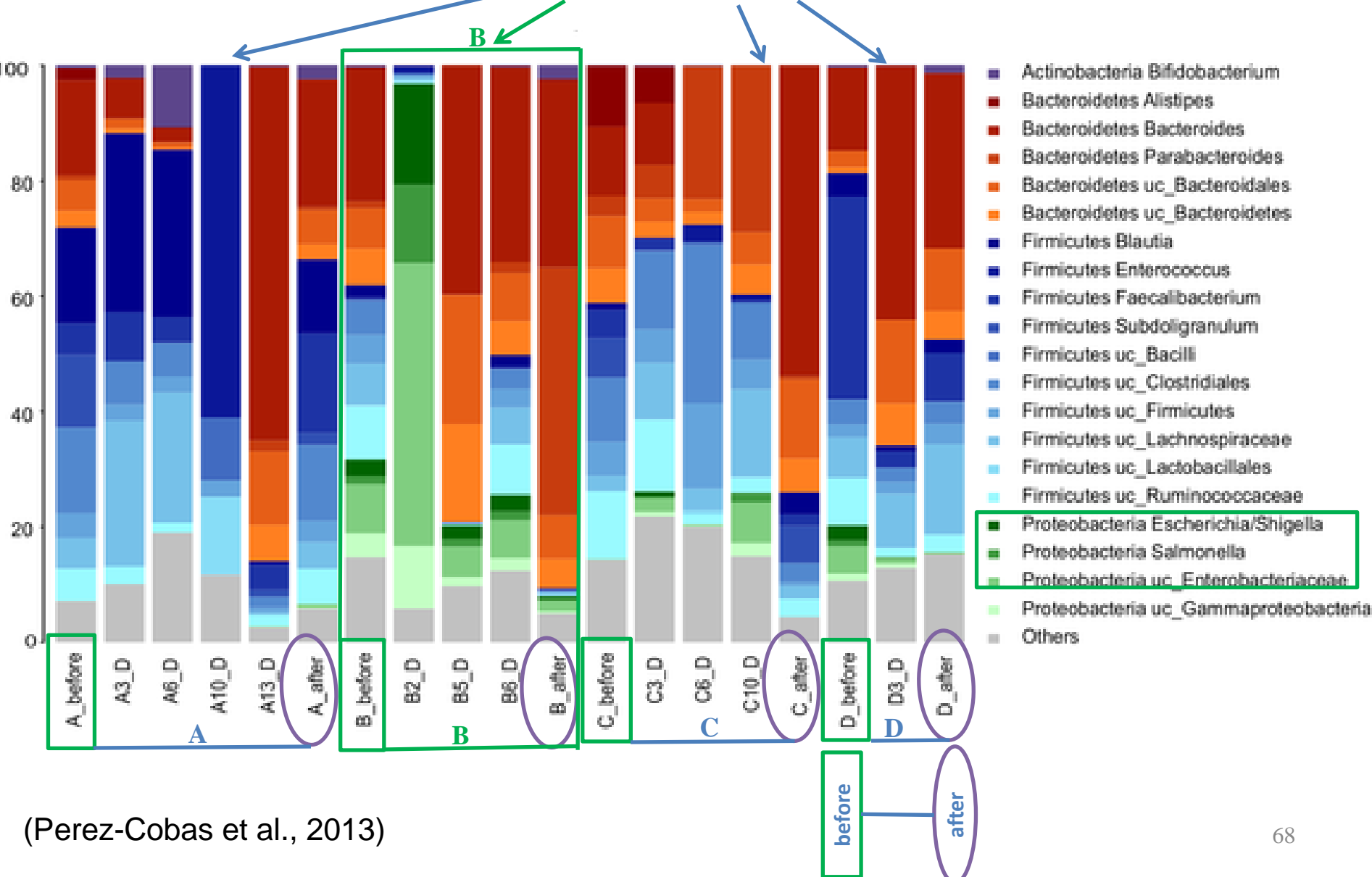
- **Colonization resistance**

- is associated with a **stable and diverse microbiota** that do not trigger gut 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)

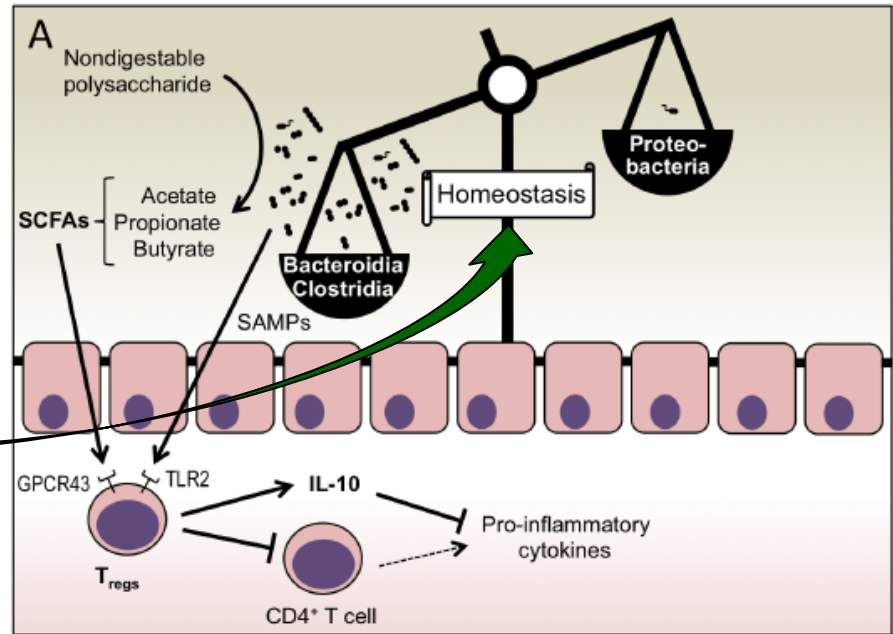
Antibiotics Shift Gut Microbiota in Four Volunteers

DYSBIOSIS

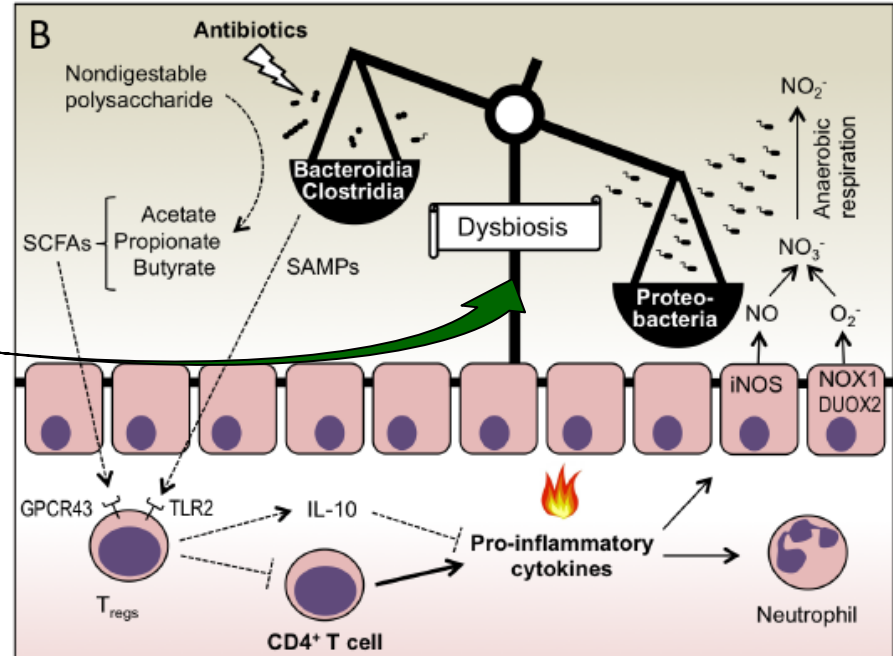


(Perez-Cobas et al., 2013)

Colonization Resistance in Homeostasis,



Disrupted in Dysbiosis



Additional Mechanistic Examples of Colonization Resistance:

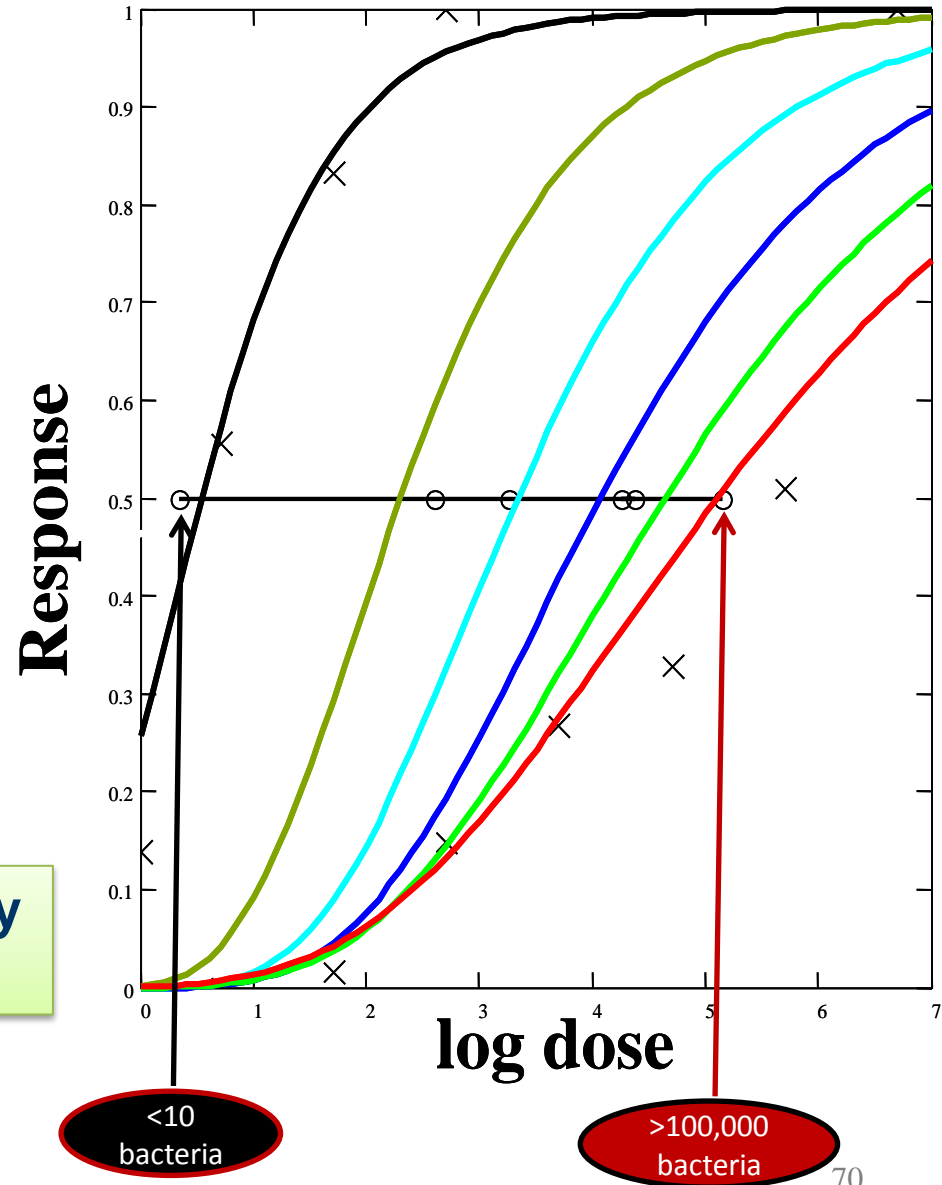
- Lawley and Walker, 2012
- Masanta et al., 2013
- Ostaff et al., 2013
- Pham and Lawley, 2014

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

Dysbiosis shifts host susceptibility five orders of magnitude!

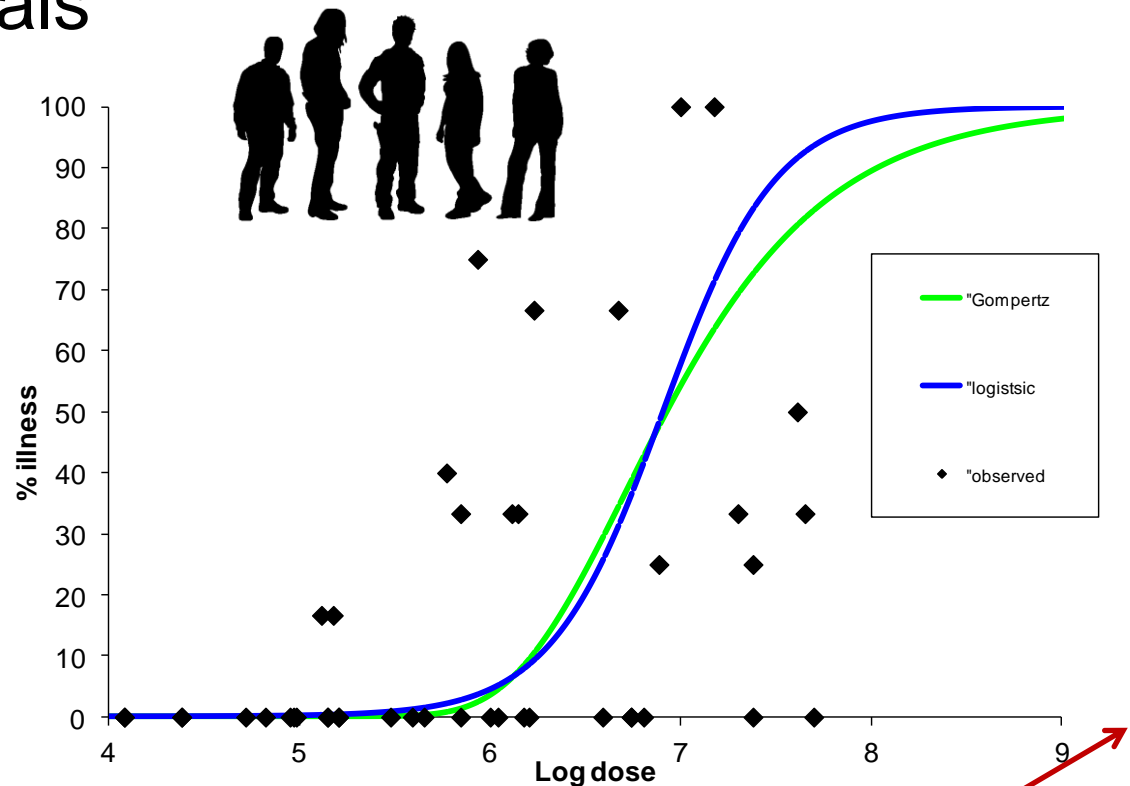
Derived by Coleman and Marks (1999, 2000) from data of Bohnhoff et al. (1954)



Salmonella Strains Administered to Humans

Human clinical trials

- anatum
- bareilly
- derby
- meleagridis
- newport

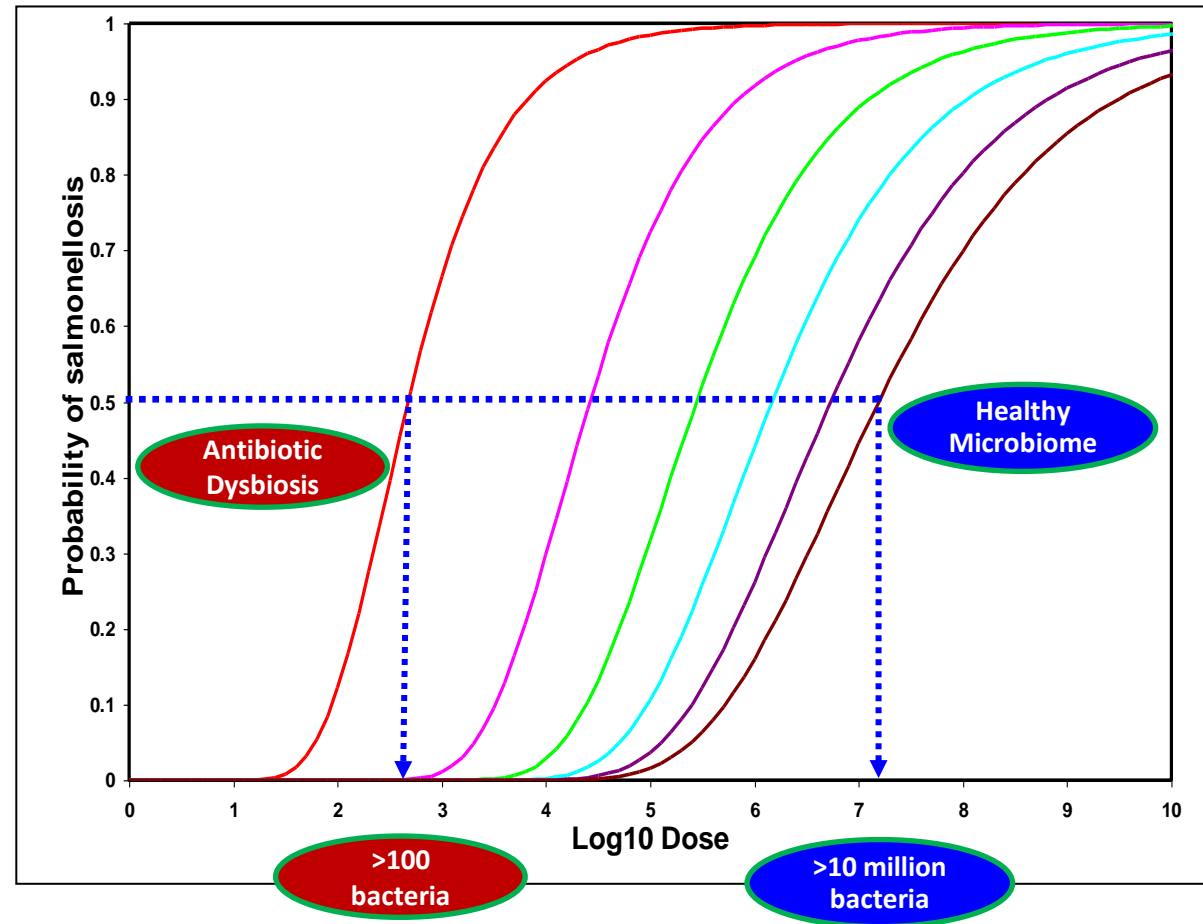


- **pullorum** (statistically significant threshold at 10⁹ bacteria)

(Coleman and Marks, 1998;
McCullough & Eisele, 1951a-d)

Predicted Magnitude of Colonization Resistance by Human GI Microbiota after Antibiotic Dysbiosis

- Half of healthy volunteers ill after dosing with $\sim 10^7$ (ten million) *Salmonella* bacteria (brown line)
- Half of volunteers with antibiotic dysbiosis likely ill after dosing with $\sim 10^2$ (>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)



(Derived by Coleman and Marks, 1999 from mouse data of Bohnhoff et al., 1954 and human data of McCullough and Eisle, 1951)

Need for Open Public Dialogue (Analytic-Deliberative Process)

Challenging Myths of Exposure Assessment Excluding Microbiota

- **Unaware of any experimental studies** in milk that substantiate the perception that fresh unprocessed certified milk is unsafe



- **Summary of experimental research** is needed to demonstrate the conditions where pathogens of concern fail to grow in certified raw milk and grow in pasteurized milk

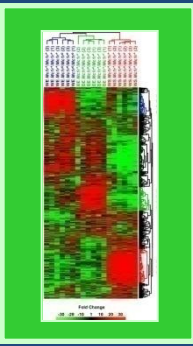
- Additional **growth studies** in **raw** and **pasteurized milk** may be needed to challenge FDA claims



Challenging Myths of Dose-Response Assessments Excluding Microbiota

- **Unaware of any studies** that **directly compare effects** in animals treated with pathogen doses administered in unpasteurized and pasteurized milk to **substantiate perception** that **pasteurized milk is safer**
- One recent study in human tissue cultured cells demonstrated a **protective effect** (lower attachment, invasion, and growth) from a **high dose of *Listeria*** when delivered in **raw milk** compared to the same *Listeria* dose delivered in **pasteurized milk** (Pricope-Ciolacu et al., 2013)
- As experimental science advances, model systems can be used to directly test and compare alternatives, **rather than assuming** that **past expert opinions** on dose-response relationships that excluded the effects of the microbiota are valid

Spatial Scales in Systems Biology and Risk

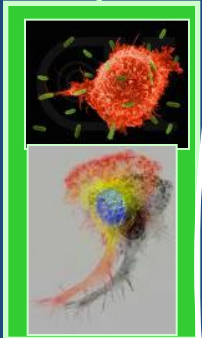


Genetic



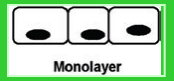
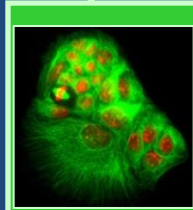
Molecular

In vitro cultures



Cellular

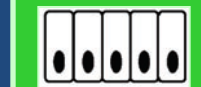
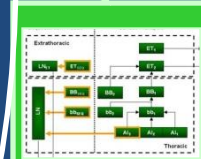
In vitro monolayers



Monolayer

Tissue

Organoid models



Intestinal tissue and organoid

Organ

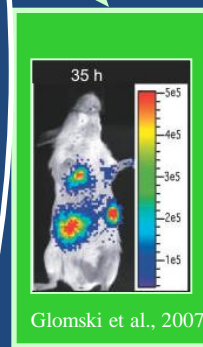
Edwards et al., 2010
innate defenses in
ex vivo **human** colon
biopsies eradicate
Campylobacter

Bereswill et al., 2011
innate defenses in
humanized mice
eradicate
Campylobacter

Dicksved et al., 2014
human fecal
microbiota associated
w susceptibility or
resistance to
Campylobacter

Archambaud et al., 2012
colonization resistance
against Lm in
rodents

Animal studies



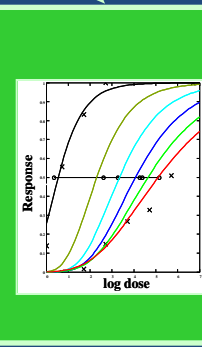
35 h

Glomski et al., 2007

Individual

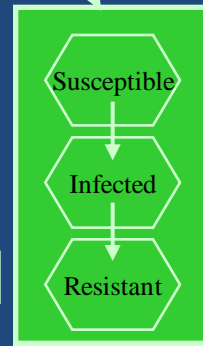
Gomes et al., 2012,
Nakamura et al. 2012,
colonization
resistance
against Lm
invasion

Host sensitivity



Subpopulation

SIR models



Population

Coherent
Dose-Response
Assessment

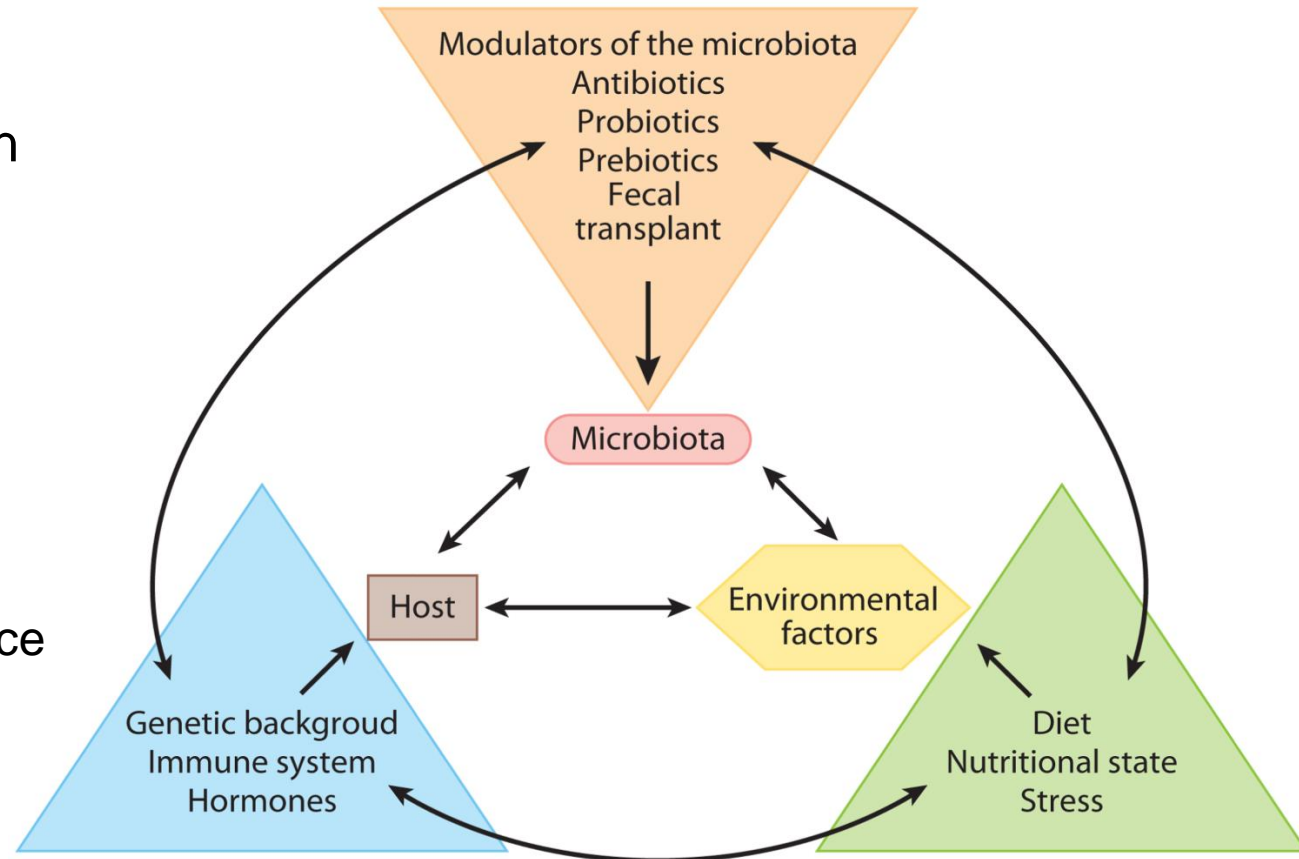
Future Evolution of Microbial
Risk Assessment

Expanded Framework of Interactions Needed for Microbial Risk Analysis

Extrinsic factors influencing composition and function of GI microbiome:

- Age
- Malnutrition
- Antibiotic use
- Probiotic use
- Dietary habits
- Geographic provenance

(Lagier et al., 2012)

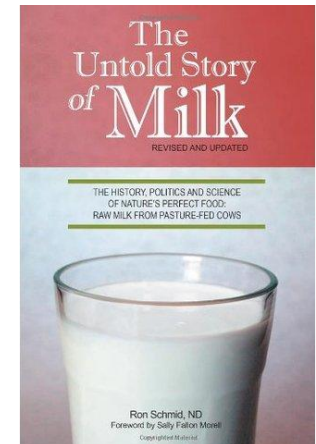
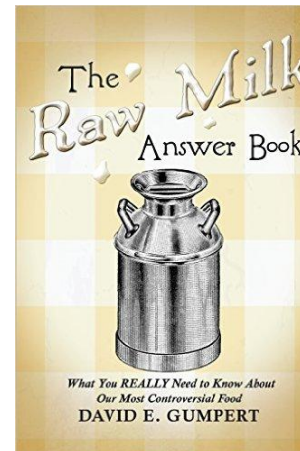


(Eloe-Fadrosh and Rasko, 2013)

Overcoming Perceptions, Beliefs, Biases in Risk Analysis for 'Fresh Unprocessed' Milk

Strong scientific evidence that raw milk is **NOT** poison and confers **health benefits** to consumers

- Loss et al., 2011, 2012, 2015 (PASTURE study, 983 infants in EU countries)
- Frei et al., 2014 (PARSIFAL study, 316 Swiss children)
- Sozanska et al., 2013 (1700 villagers in Poland)
- Montjoux-Regis et al., 2011 (55 premature infants, raw mothers milk or pasteurized donor milk in France)
- Leighton & McKinlay, 1931; Taylor, 1931; Fisher & Bartlett, 1931 (20,000 school age children in UK)



WAPF Real Milk Campaign, <http://www.realmilk.com/>,
<http://www.westonaprice.org/>

Microbial Risk Assessment Scenarios, Causality, and Uncertainty

M. E. Coleman, B. K. Hope, H. G. Claycamp, and J. T. Cohen

Difficulties establishing causality in biological systems are abundant—and they affect efforts to assess risks of importance in microbiology, such as likelihoods of infectious disease or development of antibiotic resistance. Consider the case in which von Pettenkofer openly challenged Robert Koch on whether *Vibrio cholerae* causes cholera by drinking some of the cholera bacillus (*Microbe*, May 2006, p. 223). When von Pettenkofer failed to develop symptoms of cholera, he concluded that *V. cholerae* does not cause the disease. Of course, subsequent experimental research established that this microbe causes cholera, and von Pettenkofer merely proved that exposure to the pathogen alone is not sufficient to cause this illness.

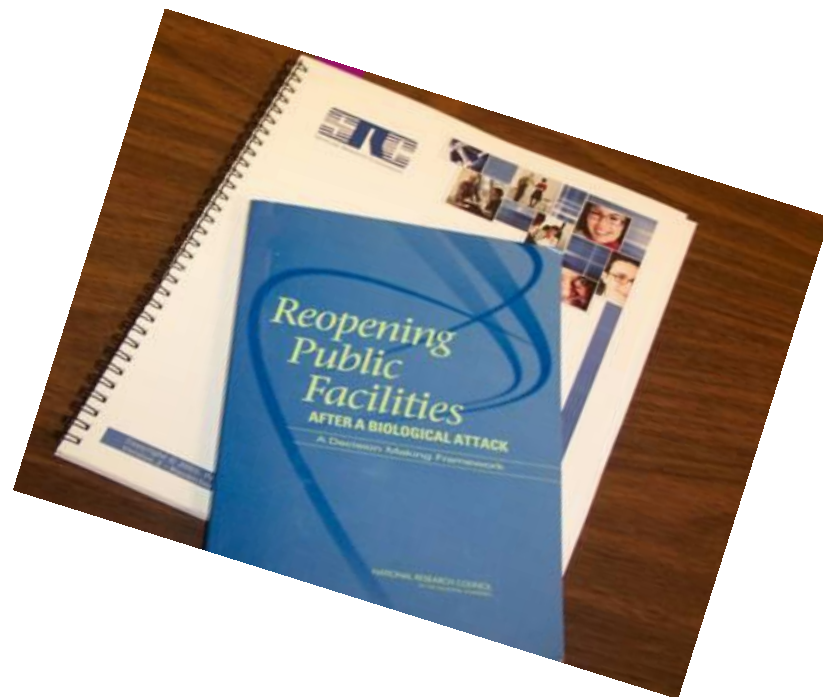
A basic principle of risk assessment is that multiple factors, including the ingested or inhaled dose of a pathogen, determine the likelihood and severity of adverse effects. More generally for both chemical and microbial hazards, there is a widely accepted framework for risk assessment that encompasses four main elements: hazard identification, exposure assessment, dose-response assessment, and risk characterization. One key point is that dose-response predictions are uncertain, as exemplified in the von Pettenkofer case.

Assessing Risk of Infectious Diseases Proves a Complex Undertaking

With our understanding of host-pathogen interactions and infectious diseases, one could pose several alternative hypotheses to explain why von Pettenkofer did not develop cholera after ingesting *V. cholerae*. For instance, possible explanatory factors include host defenses, the virulence and physiological state of organisms in the ingested dose, the environment of the flask and gastrointestinal tract, or some combination of those factors. Perhaps von Pettenkofer was resistant or highly tolerant of that pathogen. Thus, any one or several combined factors could have mitigated the biological effectiveness of his ingested dose.

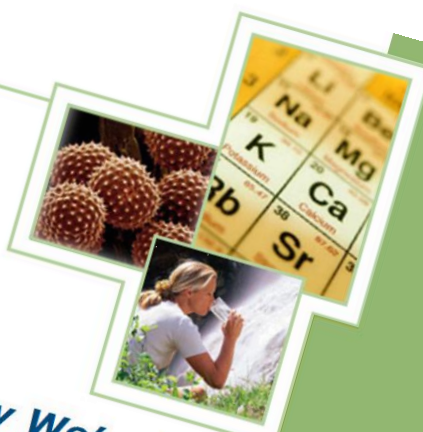
A formal dose-response assessment for cholera would present evidence for the factors that cause or control pathogenesis and virulence, typically described in terms of the infectious disease triangle (interactions among the host, pathogen, and environment). Koch could have miscalculated the dose needed to cause illness in a human because his experience was based mainly on how pathogen preparations affected animals. The von Pettenkofer example illustrates

M. E. Coleman is Senior Microbiologist at Syracuse Research Corporation, Syracuse, N.Y.; B. K. Hope is a Senior Environmental Toxicologist with the Oregon Department of Environmental Quality, Portland; H. G. Claycamp is Sr. Advisor for Risk Assessment Food and Drug Administration, Md.; and J. T. Cohen is Lecturer at Tufts University School of Medicine, Boston, Mass.



Summary

- Difficulties establishing causality are abundant, and they affect efforts to assess microbiological and other biological or chemical risks of importance.
- Risk assessments encompass four main elements: hazard identification, exposure, dose-response, and risk characterization.
- USDA assessments of how much antimicrobial resistance is attributable to food animal sources depended on possible causal pathways; however, shifting those possibilities to probability values appears to oversimplify matters and complicates efforts to understand mechanisms.
- Scientific data are inadequate for predicting potential adverse effects from infectious agents such as those responsible for bovine spongiform encephalopathy, avian flu, and diseases from intentional or accidental releases of bioterror agents.



Thresholds: Why We're Not Dead Yet!
 Peg Coleman, Senior Microbiologist
 Presented October 13, 2006 to Upstate NY SRA
 mcoleman@syrres.com

Thanks to:

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- Weston A. Price Foundation



Questions?



Contact Information:

peg@colemanscientific.org

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

<http://www.colemanscientific.org/index.html>

Groton, New York

315 729 3995 mobile



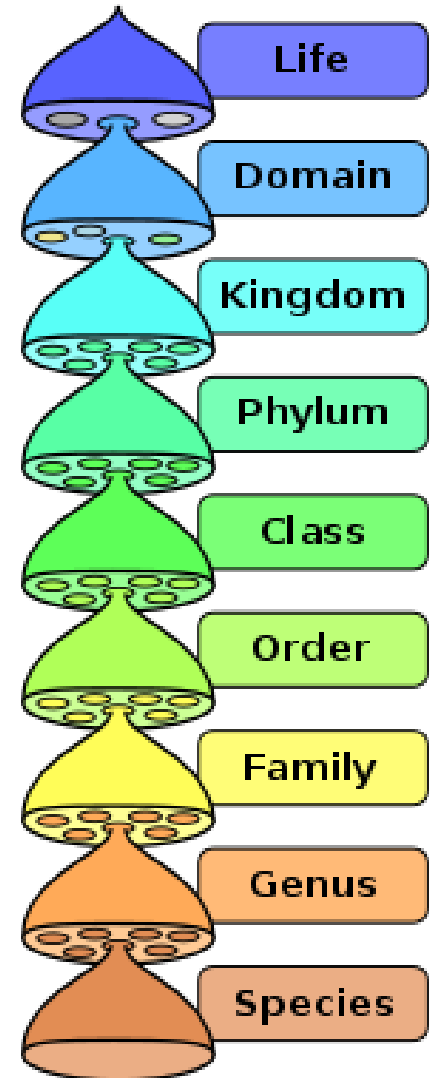
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Backup Slides

Mnemonic for Bacterial Taxonomic Ranks

First Letter	Mnemonic	Taxonomic Rank	<i>Escherichia coli</i>
D	Determined	Domain	Bacteria
K	Kind	Kingdom	Bacteria
P	People	Phylum	Proteobacteria
C	Can	Class	Gammaproteobacteria
O	Often	Order	Enterobacteriales
F	Follow	Family	Enterobacteriaceae
G	Ghostly	Genus	<i>Escherichia</i>
S	Screams	Species	<i>E. coli</i>



Human Breast and Milk Microbiome Studies

- Recent **evidence** from **multiple studies** **inconsistent** with prior assumptions
 - Healthy breast tissue and aseptically collected milk **are sterile (NOT TRUE)**
 - Milk microbiota **are contaminants (NOT TRUE)**
 - **Pasteurized** milk is **healthier** for infants **(NOT TRUE)**
- **Breast tissue microbiomes** include 7 phyla predominated by *Proteobacteria* (rare phylum in GI, oral, skin, and vagina microbiomes)
- **Breast milk microbiomes** also complex, variable over time:
 - Birth – 5 genera
 - After 1 – 6 months an increase of 11 new genera
 - Up to 700 bacterial species



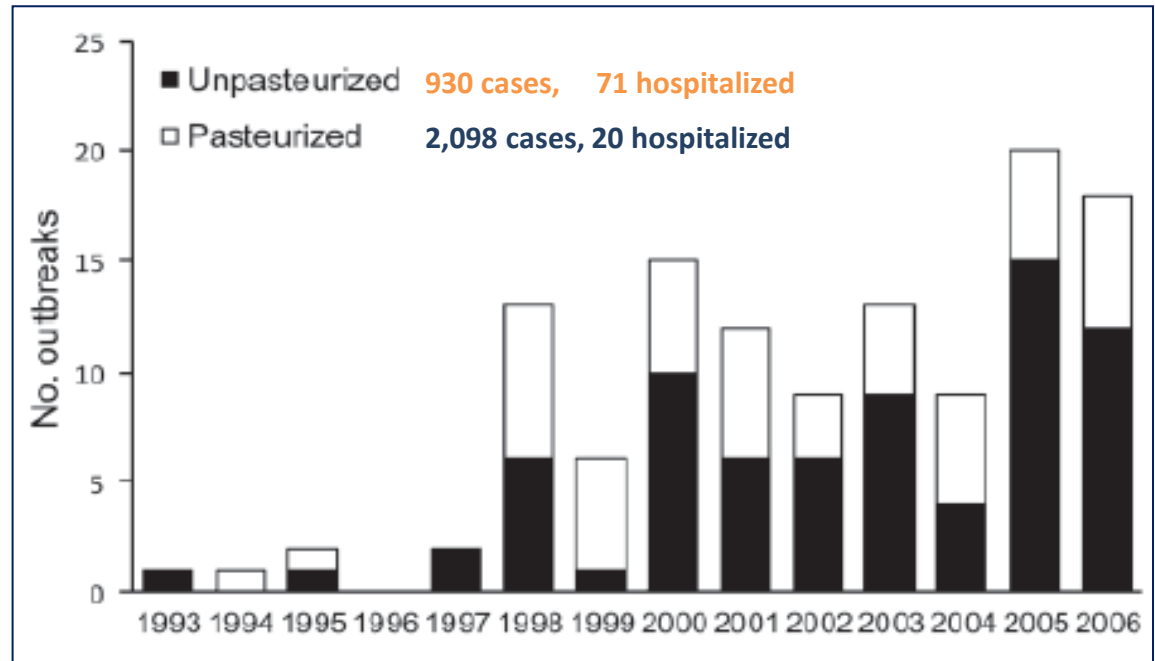
(Hunt et al., 2011; Montjoux-Regis et al., 2011; Cabrera-Rubio et al. 2012; Jost et al., 2013; Quigley et al., 2013; Sozanska et al., 2013 Urbaniak et al., 2014)

Epidemiologic Evidence from Milk Outbreaks

Limited Utility for Risk Analysis

Pathogens associated:

- *Bacillus* (pasteurized)
- *Brucella* (unpasteurized)
- *Campylobacter* (both)
- *Clostridium* (pasteurized)
- *Listeria* (both)
- Norovirus (pasteurized)
- *Salmonella* (unpasteurized)
- *Shigella* (both)
- *Staphylococcus* (both)
- STECs/VTECs (unpasteurized)



Gaps and Uncertainties

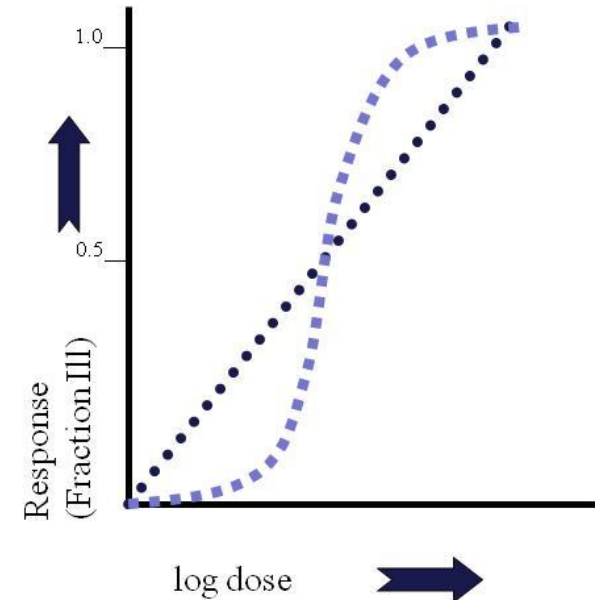
- Don't know **doses ingested** for cases of illness or those exposed who did not become ill
- **Attack rates** (#cases/#exposed) not whole story
- Repeated charges of **selective use of data** leads to **biased analyses**

(Langer et al., 2012)

Revisiting Scientific Principles for Microbial Dose-Response Assessment

- **Increasing the pathogen dose generally increases**
 - ✓ Likelihood of illness
 - ✓ Severity of illness
 - ✓ Duration of illness
- **Increasing the pathogen dose may decrease**
 - ✓ Incubation period
 - ✓ Fraction with asymptomatic illness
 - ✓ Time to mortality
- **Exposure ≠ illness (or mortality!)**

- Progression of **infection** and **illness** requires **growth** in host tissues after an incubation period (**days or weeks**) during which **host defenses** are **activated** in healthy hosts
- **Low doses may not cause illness**
 - Defenses including **GI microbiota** exerting **colonization resistance**, prevent adherence and growth of low doses of pathogens, and thus prevent **illness**



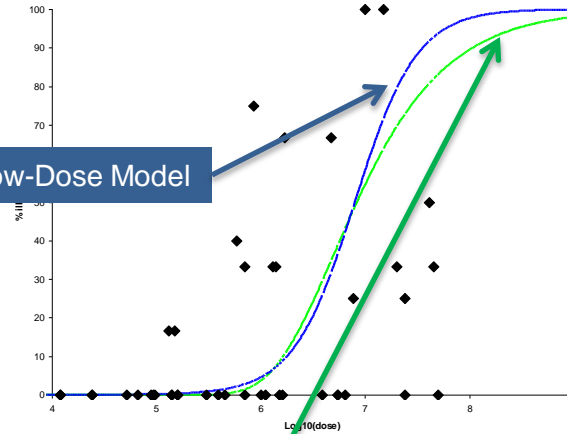
Exposures frequent and asymptomatic for **farm families** including children, even healthy six-month old baby positive for *E. coli* O157:H7 (Wilson et al., 1996; Karmali et al., 1996; Haack et al., 2003)

Human Dose-Response, Informed by Mouse Data

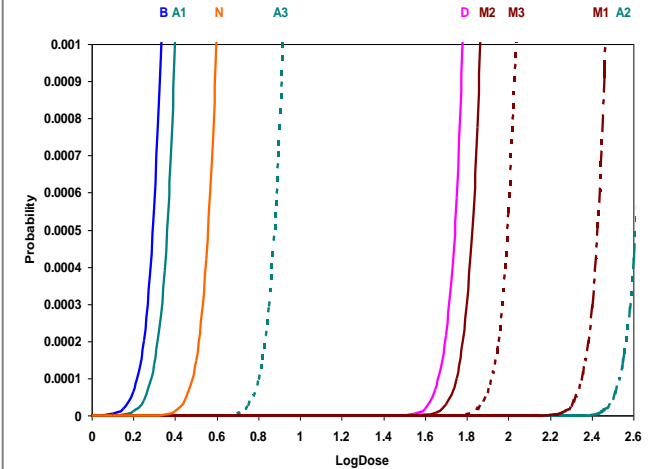
Empirical Models for Human Salmonellosis

Linear Low-Dose Model

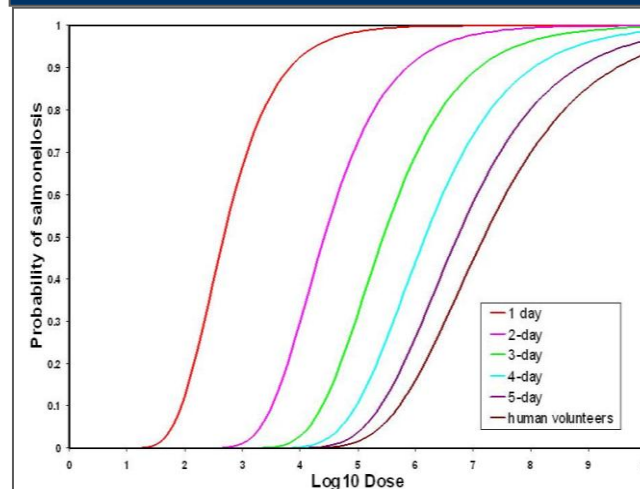
Sublinear Low-Dose Model



Family of Empirical Models for Strain Variability



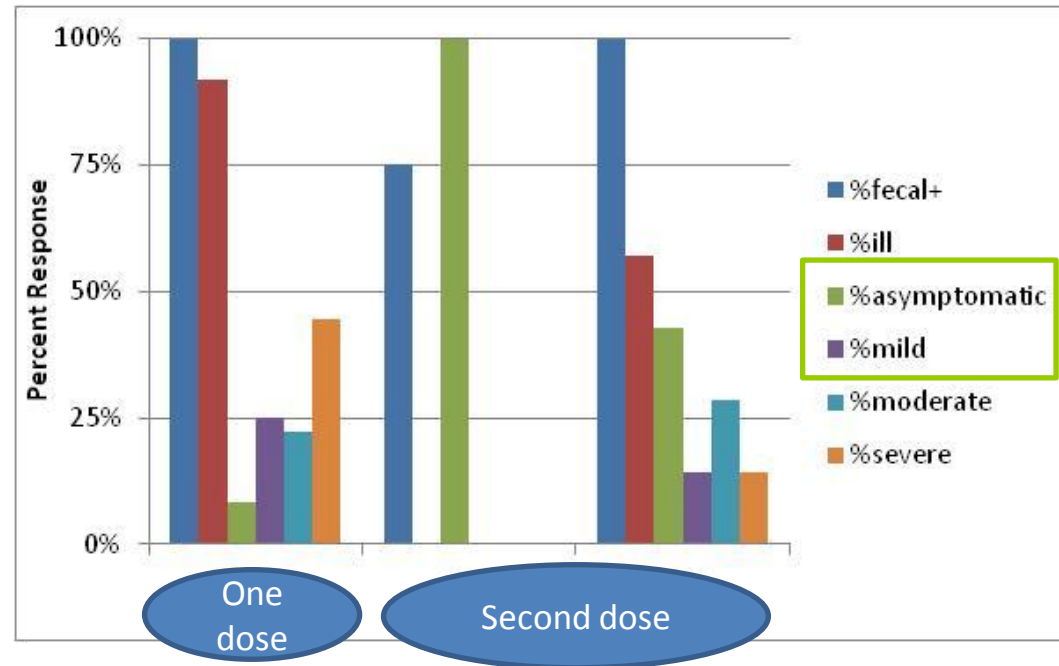
Family of Empirical Models for Host Variability



Campylobacter Administered to Soldiers

Factors Influencing Travelers Diarrhea

- **Innate immunity** in volunteers, even after **one high dose** (10^9 or **one billion bacteria**)
- **Adaptive immunity** (antibodies) protect after **second dose** over time, all asymptomatic in short term
- **Previous antibiotic** treatment
- Fatigue, physical and psychological stress
- Boredom with ready-to-eat meals
- Failure of **public health advice** to prevent **travelers' diarrhea**



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