Exploring Disagreements Regarding Health Risks of Raw and Pasteurized Human & Bovine Milk

Society for Risk Analysis Annual Meeting
Peg Coleman
December 8, 2014
Dedications

• To the MD risk assessor who chooses to purchase 4 gallons of raw milk for his family of five every week

• To my upstate NY neighbors and farm families who choose to consume raw milk

• To 43 states including NY state who permit sale of raw milk from licensed dairies

• To advocates of banning and permitting sale of raw milk in hopes that they have the opportunity to participate together in analytic deliberative process on this issue
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 activated in healthy hosts

Low doses may not cause illness
  - Innate defenses (including GI microbiota) and repair mechanisms may prevent adherence or clear pathogens
Questions about Risk from Recent US Milk-Associated Outbreaks
(All Nonfatal, Some Hospitalizations)

Pathogens associated:

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

![Graph showing the number of outbreaks from 1993 to 2006 for pasteurized and unpasteurized milk products.](image)

Langer et al., 2012
N=56 milk of 121 dairy

Note: Other dairy commodities associated with outbreaks can include butter, cheeses, particularly soft styles, and fermented milk products.
• Expanding knowledge from ‘omics research characterizing indigenous ‘microbiomes’ of milk from humans and ruminants

• Risks, assumptions, data for assessments for listeriosis fatalities from consumption of raw and pasteurized bovine milk

• Incorporating microbiome research into NextGen Microbial Risk Assessment, highlighting key terms:
  – ‘Colonization Resistance’
  – ‘Dysbiosis’
Healthy Human Microbiomes

• Human Microbiome Project (2007-present)
  – Culture-based, culture-independent, genetic methods (‘omics, 16S rRNA sequences)
  – Ecological/statistical methods (e.g., clustering/spatial analysis, diversity indices)

• Results continue to challenge old dogma:
  – Many healthy human tissues were presumed sterile (free of microorganisms)

Cho and Blaser, 2012
Diversity in Healthy Human Microbiomes

Microbiome communities include:

• Up to **7 of 29 known bacterial phyla** (major taxonomic lineages) and hundreds or thousands of ‘operational taxonomic units’ and species

• Variable density, associated with particular anatomical niches

• Aerobes, facultative aerobes, anaerobes

• Various classes of relationships with hosts:
  – Predominantly **commensal** (colonize without causing signs/symptoms of disease, potential for mutual benefit or symbiosis)
  – Some **opportunistic pathogens** associated with disease in immunocompromised hosts
  – Few **frank pathogens** at >0.1% abundance (NIAID class A-C)

• ‘Permanent’ stable colonizers as well as transitory organisms that do not colonize consistently over time, grow, and maintain density in relation to other taxa present
Relatedness of Human Microbiota

- Not random associations, some niches with ‘core’ taxa present or dominant in most individuals
- More similarity between individuals for same niche than between niches of an individual
- Stability and resilience high, but influenced by various perturbations

Cabrera-Rubio et al., 2012
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)

• **Breast microbiomes** include 7 phyla predominated by *Proteobacteria* (rare phylum in GI, oral, skin, and vagina microbiomes)
  – *Enterobacteriaceae, Staphylococcus, Listeria welshimeri, Propionibacterium, Pseudomonas*
  – *Bacillus, Acinetobacter, Enterobacteriaceae, Pseudomonas, Staphylococcus, Propionibacterium, Comamonadaceae, Gammaproteobacteria, Prevotella*

• **Milk microbiomes** also complex, variable over time and conditions:
  – *Weisella, Leuconostoc, Staphylococcus, Streptococcus, Lactococcus* at birth
  – Above genera plus increasing presence of other genera including *Veionella, Leptotrichia, Prevotella, Serratia, Corynebacteria, Propionibacterium, Pseudomonas, Bacteroides, clostridia, Enterococcus, Acinetobacter* after 1 - 6 months
  – Up to 700 bacterial species

Hunt et al., 2011; Cabrera-Rubio et al. 2012; Jost et al., 2013; Quigley et al., 2013; Urbaniak et al., 2014
Recent Characterizations of Milk Microbiota

Bacterial Families in Human Milk

Cabrera-Rubio et al., 2012
Recent Characterizations of Milk Microbiota

Bacterial Genera in Human Milk

Cabrera-Rubio et al., 2012
Recent Characterizations of Milk Microbiota
Bacterial Genera in Human Milk over Time by Individual

Hunt et al., 2011
Comparing Milk Microbiota by Host, Method
Culture Dependent (CD), Next Generation Sequencing (NGS)

Quigley et al., 2013
Risks, Assumptions, Data
Risk Assessment Results on Listeriosis from Milk

• FDA-FSIS, 2003
  – US consumers exposed to low to moderate levels of *Listeria monocytogenes* in foods (23 categories investigated) on a regular basis
  – Model predicts:
    » **Pasteurized milk**
      – High risk, 90.8 fatal cases predicted per annum
      – Moderate risk, $10^{-9}$ fatal cases per serving
    » **Unpasteurized milk**
      – Moderate risk, 3.1 fatal cases predicted per annum
      – High risk, $7 \times 10^{-9}$ fatal cases per serving

• Latorre et al., 2011
  – Median risk of listeriosis fatalities per serving to US consumers of **unpasteurized milk** on farms or sold by permitted dealers
    » Bulk tanks, $7 \times 10^{-7}$ fatal cases per serving
    » On-farm stores, $4 \times 10^{-5}$ fatal cases per serving
    » Retail where permitted, $5 \times 10^{-5}$ fatal cases per serving
    » Farm families and staff, $1 \times 10^{-7}$ fatal cases per serving

Neither observed!
Bias in “What if” Growth Scenarios for *Listeria* in Milk

• FDA, 2003

  – Simple exponential growth as measured in milk, not accounting for milk microbiota
  – Scenarios under various refrigerated storage intervals (0.5 to 15 days, most likely interval 3-5 days for pasteurized milk and 0.5 to 10 days, most likely interval 2-3 days for unpasteurized milk)

• Latorre et al., 2011

  – Prevalence data (6.5%, 2.1%, 1.3%, 35.3%, 25.4%) were used for different scenarios
  – Initial concentrations simulated from cumulative distribution from 0.04 to 150 CFU/mL *Listeria monocytogenes* in bulk tank milk
  – Simple exponential growth modeled in pasteurized milk, not accounting for milk microbiota

Low numbers of pathogens subject to stochastic growth, lag, reduced rate of growth, and reduced maximum population density in competition with dense milk microbiota predominated at refrigeration temperatures by faster growing non-pathogenic *pseudomonads*
## Model Competition with Milk Microbiota

<table>
<thead>
<tr>
<th>Study</th>
<th>Numbers of Positives (range; mean; median) in CFU/mL</th>
<th>Standard Plate Count</th>
<th>Listeria monocytogenes</th>
<th>STEC/VTEC</th>
<th>Salmonella spp.</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D’Amico et al., 2008</strong></td>
<td></td>
<td><strong>Farmsted dairies</strong></td>
<td>N=62</td>
<td>62</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(10 to 10^5; 4.9x10^4; 7.0 x10^2)</td>
<td>&lt;1</td>
<td>-</td>
<td>Unspecified; 250; &lt;1</td>
</tr>
<tr>
<td><strong>Total Viable Count</strong></td>
<td></td>
<td></td>
<td><strong>Listeria monocytogenes</strong></td>
<td>23</td>
<td>30</td>
<td>5 - 33</td>
</tr>
<tr>
<td><strong>Jackson et al., 2012</strong></td>
<td></td>
<td><strong>Commercial dairy silos</strong></td>
<td>N=184</td>
<td>184</td>
<td>23</td>
<td>3 to 93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(7x10^2 to 5x10^5; 4.2 x10^4)</td>
<td>&lt;0.006 to 29</td>
<td>&lt;0.006 to 1.1</td>
<td>3 to 93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.65</td>
<td>0.19</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.12</td>
<td>0.26</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.006 to 60</td>
<td>&lt;0.006 to 60</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.75</td>
<td>0.12</td>
<td>0.12</td>
</tr>
</tbody>
</table>

**Temperature (°C)**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.09</td>
<td>No Growth</td>
<td>No Growth</td>
<td>No Growth</td>
</tr>
<tr>
<td>4</td>
<td>0.11</td>
<td>0.01</td>
<td>No Growth</td>
<td>No Growth</td>
</tr>
<tr>
<td>10</td>
<td>0.24</td>
<td>0.07</td>
<td>0.07</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**Optimal growth conditions in broth (Coleman et al., 2003)**
Dose-Response Models for Listeriosis in Milk

• FDA, 2003
  – Complex adjustments of mouse mortality model (shifted 8-13 orders of magnitude) in order to anchor fatality predictions over 23 commodities for total expectation of 91 fatalities per annum in US population

• Latorre et al., 2011
  – Simple exponential dose-response model for fatalities from hospital outbreak associated with contaminated butter

Overprediction bias for morbidity (GI illness)
Alternative Dose-Response Models and Approaches

• Considering available animal studies for listeriosis and providing rationale for extrapolations of host species and endpoint
  – Gerbil study on pathology, invasion, morbidity, and mortality
    • Roulo et al., 2014
  – Rhesus monkey studies on pathology, immune modulators, stillbirth, mortality
    • Smith et al., 2003, 2008

• Accounting for Lm strain effects (Chen et al, 2011; see Pouillot talk T4-J)

• Other agents:
  – Accounting for adaptive immunity (Havelaar et al., 2014)
  – Accounting for innate immunity, including protective effects of GI microbiota in healthy humans (Coleman and Marks, 1999, 2000)
Microbiome Research and Next Gen Microbial Risk Assessment
GI Microbiome in Health

• **Food-Borne Microbes**: Shaping the Host Ecosystem
  • Preface cites estimate of $10^{10}$ (10,000,000,000!) microorganisms as daily dietary consumption, most beneficial/harmless **commensals** and **few pathogens** (Jaykus et al., 2009)

  • GI microbiome includes consortia of >40,000 microorganisms
  • $10^{14}$ (100,000,000,000,000!) microorganisms typically present in human colon
  • Complex spatial and temporal gradients
  • **NOT** homogeneous well-mixed chemostats of nutrient media
Including Microbiota in NextGen Microbial Exposure Assessments

• Unaware of any experimental studies that measure growth of any pathogen in raw and pasteurized milk at low initial densities observed in monitoring studies (1, 10, 100 CFU/mL)

• Experimental research is needed to demonstrate the boundary conditions for growth of pathogens in the presence of microbiota of milk and other foods, rather than assuming optimal growth conditions of traditional predictive microbiology experiments in culture broth apply to non-sterile foods

• Evidence from multiple papers (most recent Alonso et al., 2014) on stochastic not deterministic growth from single bacterial cells
Including Microbiota in NextGen Microbial Dose-Response Assessments

• Unaware of any studies that measure a range of pathogen doses administered in unpasteurized and pasteurized milk and GI responses in any host system to substantiate perception that pasteurized milk containing low observed levels of pathogens is safer

• Lower adhesion, invasion, and proliferation of Lm strains at $10^8$ cfu/mL in raw milk (black) than pasteurized milk (gray) and buffer (white) using human intestinal epithelial cell (Caco-2) assay (Pricope-Ciolacu et al., 2013)

• Influence of symbiont on time- and dose-dependent models demonstrated in another pathosystem (Pessoa et al., 2014)

• Experimental model systems are advancing to the stage where definitive scenarios and studies can be designed and tested, rather than assuming that past expert opinions on dose-response relationships are valid in the presence of a healthy GI microbiota

• Additional examples relevant to GI microbiome influences follow
Perturbation of GI Microbiome (Dysbiosis)

• Knowledge of the microbiota in health and disease transforming science, medicine, taxonomy, particularly for most studied system in humans, GI tract

  – Evidence of **tolerance** of indigenous microbiota that contribute to immune development and homeostasis

  – Mechanisms of **colonization resistance** by indigenous microbiota (commensal or symbiotic associations) limiting growth of transients and pathogens until perturbation (e.g., antibiotic administration)

  – Studies on use of **probiotics** (live cultures), **prebiotics** (substrates for live cultures), **synbiotics** (live cultures and substrates) during antibiotic treatment to minimize overgrowth of *C. difficile* and other opportunistic pathogens

  – Success with **fecal transplantation** in inflammatory bowel disease patients

• Influence of GI microbiota diversity on health

  – **Dysbiosis**, perturbations that decrease diversity of GI microbiota associated with obesity, infections of *C. difficile* and other pathogens, inflammatory bowel disease

  » *C. difficile* cases lose *Bacteroides, Lachnospiraceae*, and *Ruminococcaceae* from their GI microbiota, causing **dysbiosis**, loss of normal ‘**colonization resistance**’
Colonization Resistance in Homeostatis, 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

Spees et al., 2013
Scientific Principles for NextGen 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 activated in healthy hosts

- Low doses may not cause illness
  - Innate defenses (including GI microbiota exerting colonization resistance) prevent adherence and growth of low doses of pathogens

Exposures frequent and asymptomatic for farm families including children, even healthy six-month old baby positive for O157:H7 (Wilson et al., 1996; Karmali et al., 1996; Haack et al., 2003)
Magnitude of Colonization Resistance by Murine GI Microbiota: \(10^5\)!

- Normal animal challenges with increasing doses of *Salmonella enteritidis* (red line), ID\(_{50}\) ≈ \(10^5\)

- **Antibiotic** 1 day before challenge disrupts colonization resistance and increases susceptibility (black line), ID\(_{50}\) < 10

- Microbiota recovers within 5 days (bright green line) to normal magnitude of colonization resistance

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

**Dysbiosis shifts host susceptibility five orders of magnitude!**
Predicted Magnitude of Colonization Resistance by Human GI Microbiota after Antibiotic Dysbiosis

- **Full symbiosis** in healthy human volunteers challenged with increasing doses of *Salmonella* spp. (brown line), ED$_{50} > 10^7$

- **Full dysbiosis** at day 1 after antibiotic administration (red line), ED$_{50} > 10^2$

- Indirect evidence of $10^5$ magnitude of colonization resistance (murine and human data)

Derived by Coleman and Marks, 1999 from murine data of Bohnhoff et al., 1954 and human data of McCullough and Eisle, 1951
Magnitude of Colonization Resistance for Tularemia in Primates by Route: $10^6$

- Human $ED_{50(\text{fever})}$ is $10^7$ for *Francisella tularensis* by oral route (Hornick et al. 1966)

- Human $ED_{50(\text{fever})}$ is $10^1$ (N=118) for *F. tularensis* by inhalation route (Anno et al., 1998, 2005; McClellan et al., in preparation for *Military Medicine*)

- Colonization resistance exerted by higher density/diversity of GI microbiota likely to account for a large portion of the $10^6$ difference between oral and inhalation routes in humans

- Results for rhesus monkey studies consistent with $10^6$ difference in $LD_{50}$s between oral and inhalation routes observed in humans (Day and Berendt, 1972; Eigelsbach et al. 1965)

Reports with detailed analyses FOUO documents available from Brandolyn Thran, USAPHC
Expanded Framework of Interactions Needed for NextGen Microbial Risk Assessment

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
Spatial Scales in Systems Biology and Risk

Genetic
Molecular
Cellular
Tissue
Organ
Individual
Population
Subpopulation

Coherent Dose-Response Assessment

Next Generation Microbial Risk Assessment

In vitro cultures

In vitro monolayers

Organoid models

In vivo studies

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

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

Host sensitivity

SIR models

Susceptible
Infected
Resistant
Population

Intestinal tissue and organoid

Glomski et al., 2007

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

31
Experimental Models for Innate Immunity Influenced by GI Microbiota and Diet

• Initial stage of pathogenesis after ingestion of *Campylobacter* is overcoming colonization resistance (Masanta et al. 2013)

• Idealized *in vitro* experimental test system incorporating physiological gradients and spatial and temporal phenomena controlling GI microbiota in health and dysbiosis (Fritz et al., 2013)

Fritz et al., 2013
Assessing and Managing Perceived Risks

• Need for incorporating microbiota into NextGen microbial risk assessment, both in exposure assessment and dose-response assessment

• Balanced analyses needed to support pasteurization policies for human breast milk and ruminant milk

• Formal analytic deliberative process on assumptions, data, and models for risk assessments associated with milk and other non-sterile foods valuable

  – Deeper analysis of perceived risks AND benefits essential

  – Populations and conditions associated with baseline and higher risk identified

  – Need for increased transparency and acknowledgement of biases, uncertainties, and influence of assumptions on estimated risks
Questions?

Interested in engaging in analytic deliberative process? Leave me a business card or email me at peg@colemanscientific.org
Backup Slides
Requirements for Selling Raw Milk in NY State

• Brucellosis ring test

• Tuberculosis test for each animal

• Quality Milk Production Services (QMPS) program
  – Each animal tested for *E. coli* and pathogens including *Staphylococcus aureus*

• Monthly milk sample tested for coliforms and pathogens including *Salmonella, Listeria, E. coli O157:H7, Campylobacter, Staphlococci*

• Satisfactory farm water test

• Farm inspections at least twice a year
  – Sanitary conditions
  – Health of cows
  – Health of individuals working on farm
Microbial and Immunological Signatures of Intestinal Colonization Resistance

Lawley and Walker, 2012
GI Microbiome Effects in Homeostasis and Perturbation (Dysbiosis)

Ostaff et al., 2013
Homeostasis vs Dysbiosis in GI Microbiota

Pham and Lawley, 2014
## Disruptions of GI Microbiota Associated with Noninfectious Diseases

<table>
<thead>
<tr>
<th>Noninfectious disease</th>
<th>Names of bacteria altered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 diabetes</td>
<td>Decrease in <em>Lactobacillus</em> spp.</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>Increase in <em>Bacillus</em> spp. and <em>Lactobacillus</em> spp.</td>
</tr>
</tbody>
</table>
| Obesity                                      | Increase in *Bacteroidetes*  
Decrease (to low numbers) in *Bifidobacteria* spp. |
| Inflammatory bowel disease (including Crohn’s disease and ulcerative colitis) | Increase in *Enterobacteriaceae*  
Decrease in *Bacteroidetes* and certain *Firmicutes* |
| Irritable bowel syndrome                     | Twofold increase in *Firmicutes* compared to *Bacteroidetes* with increase in *Clostridia* spp. and decrease in *Bifidobacteria* spp. |
| Celiac disease                               | Increase in *Lactobacillus* spp., *Bacteroides* spp., *Staphylococcus* spp., and *E. coli*.  
In some cases levels of *Bifidobacteria* spp. increase, while there is reduction in some cases.  
In children, there is increase in *Firmicutes* and low levels of *Bacteroidetes*. |

Lawley and Walker, 2012
## Common Constituents of GI Microbiota

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Brief Description</th>
<th>Commonly detected constituent genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinobacteria</td>
<td>Gram-positive, typically obligately anaerobic or microaerophilic. Some genera most abundant in infants</td>
<td>Atopobium, Bifidobacterium, Collinsella, Eggerthella</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Gram-negative bacilli, typically obligately anaerobic. Often abundant in the gut microbiota</td>
<td>Alistipes, Bacteroides, Barnesiella, Parabacteroides, Prevotella</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Gram-positive, typically obligately anaerobic. Often abundant in the gut microbiota, and typically highly diverse. Majority of constituent species have yet to be cultured in the laboratory</td>
<td>Lachnospiraceae family: Anaerostipes, Blautia, Butyribrio, Coprococcus, Dorea, Lachnospira, Roseburia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ruminococcaceae family: Anaerotruncus, Coprobacillus, Faecalibacterium, Ruminococcus, Subdoligranulum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other Firmicutes Acidaminococcus, Dialister, Enterococcus, Finegoldia, Holdemania, Lactobacillus, Megasphaera, Phascolarctobacterium, Streptococcus, Veillonella</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*Segmented filamentous bacteria (note: these bacteria do not appear to inhabit the human intestine)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alcaligenes, Bilophila, Campylobacter, Desulfovibrio, Enterobacter, Escherichia, Hafnia, Helicobacter, Klebsiella, Oxalobacter, Parasutterella, Proteus, Sutterella</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Akkermansia, Fusobacterium, Victivallis</td>
</tr>
</tbody>
</table>

| Proteobacteria | Gram-negative, mainly facultatively anaerobic species. Includes many pathogenic species                                                                                                                         |                                                                                                                                                      |
|               | Typical less abundant members of the gut microbiota                                                                                                                                                           |                                                                                                                                                      |

Lawley and Walker, 2012
Relative Abundance of Operational Taxonomic Units (OTUs) in GI Microbiota of Weaned Members of a Family

Schloss et al., 2014
Phylum-level Variation in GI Microbiota

Eloe-Fadrosh and Rasko, 2013
N=648 fecal samples NIH Human Microbiome Project
Human Microbiota at Phylum and Genus Levels

Grice and Segre, 2012
Diversity at Different Phylogenetic Scales

Lozupone et al, 2012
Figure 4. Phylogenetic profile of bacterial genera for uninfected and *M. tuberculosis* H37Rv infected mice. Stacked bar charts for uninfected and H37Rv-infected mice of the 16 main genera identified based on ≥1% abundance present in at least two samples. Unclassified sequences are not shown. The black colored bar along x-axis indicates the five uninfected mice, while the red colored bar indicates mice infected with H37Rv.

doi:10.1371/journal.pone.0097048.g004
Uncertainties for Human Salmonellosis

Empirical Models for Human Salmonellosis

Family of Empirical Models for Strain Variability

Family of Empirical Models for Host Variability

Linear Low-Dose Model

Sublinear Low-Dose Model
Serotypes Administered to Humans

- *Salmonella* serotypes administered in clinical trials to human volunteers
  - anatum
  - bareilly
  - derby
  - meleagridis
  - newport
  - pullorum (statistically significant threshold at $10^9$ CFU)
Human Campylobacteriosis DR Data

- Black et al., 1988
  - 111 volunteers
  - Two *Campylobacter jejuni* strains administered
    - 81-176
    - A3249
  - Endpoints measured
    - Fecal positive at unspecified interval after dosing
    - Immunoglobulins
    - Fraction with fever and diarrhea
    - Diarrheal volume

- More recent dataset from Tribble et al., 2010
  - 111 volunteers
  - One *Campylobacter jejuni* strains administered (81-176)
Campylobacteriosis in humans

(Black et al., 1988)

<table>
<thead>
<tr>
<th>strain</th>
<th>dose</th>
<th># volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>81-176</td>
<td>1.0E+06</td>
<td>7</td>
</tr>
<tr>
<td>81-176</td>
<td>2.0E+08</td>
<td>10</td>
</tr>
<tr>
<td>81-176</td>
<td>2.0E+09</td>
<td>22</td>
</tr>
<tr>
<td>A3249</td>
<td>8.E+02</td>
<td>10</td>
</tr>
<tr>
<td>A3249</td>
<td>8.E+03</td>
<td>10</td>
</tr>
<tr>
<td>A3249</td>
<td>9.E+04</td>
<td>13</td>
</tr>
<tr>
<td>A3249</td>
<td>8.E+05</td>
<td>11</td>
</tr>
<tr>
<td>A3249</td>
<td>1.E+06</td>
<td>19</td>
</tr>
<tr>
<td>A3249</td>
<td>1.E+08</td>
<td>5</td>
</tr>
<tr>
<td>A3249,B</td>
<td>1.E+08</td>
<td>4</td>
</tr>
</tbody>
</table>
Uncertainties for Human Campylobacteriosis

- Low predictability for unknown strains in food/water
Host Risk Factors Important in Likelihood and Severity of Disease

- **Immunity** from previous exposures (naïve volunteers, short term veterans (STV), long term veterans (LTV) for $10^9$ dose groups in Tribble campylobacteriosis study (2010)
- Fatigue and physical stress
- 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
Pathogen Factors Important

Dose-Likelihood

Dose-Severity

EPEC

Percent Response

Average Diarrheal Volume

log10 Dose

log10 Dose

log10 Dose

Percent Response