It's a mad, mad, mad, mad intestinal tract.
Continued Adventures in Enteric Diseases

Salmonella
Vibrio cholera
Campylobacter jejuni
Helicobacter pylori
Salmonella

**Characteristics:**
- Gram-negative rod
- Motile

**Diseases:**
- Fecal oral spread
- **Typhoid fever** (symptoms 1 wk to 1 month, high fever due to systemic infection)
- **Gastroenteritis** (symptoms 6 to 24 h after ingestion—nausea, vomiting, diarrhea)

**Reservoirs:**
- Human carriers (especially asymptomatic carriers)
- Livestock animals, reptiles
Salmonella serotypes are host adapted

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Host</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhi</td>
<td>Humans</td>
<td>Typhoid fever</td>
</tr>
<tr>
<td>Paratyphi</td>
<td>Humans</td>
<td>Paratyphoid fever</td>
</tr>
<tr>
<td>Gallinarum</td>
<td>Poultry</td>
<td>Fowl typhoid</td>
</tr>
<tr>
<td>Pullorum</td>
<td>Poultry</td>
<td>Pullorum disease</td>
</tr>
<tr>
<td>Enteriditis</td>
<td>Rodents</td>
<td>Murine typhoid</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>Rodents</td>
<td>Murine typhoid</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>Bacteremia</td>
</tr>
<tr>
<td>Dublin</td>
<td>Cattle</td>
<td>Bacteremia</td>
</tr>
<tr>
<td>Cholerasuis</td>
<td>Swine</td>
<td>Bacteremia</td>
</tr>
</tbody>
</table>
Historical *Salmonella*

**Biochemical Assays**
(don’t ferment lactose, produce $H_2S$)

**Widal Test** (antibody agglutination)
**Kauffman-White Schema**

**Phage Typing**
(>300 definitive phage types for Typhimurium alone)

**MLEE and DNA Hybridizations**
(phylogeny, not just classification – share 85-100% of genetic Information with up to 500kb being unique to a particular serovar)

>Somatic O-antigen
Flagellar H-antigen
Capsular Vi-antigen

>2000 (some say >2300) serovars identified to date
E. coli API strip

Salmonella API strip

Lac\(^-\), forms H\(_2\)S, Produces gas during glucose fermentation.
Salmonella Gastroenteritis

• Symptoms begin 1 to 2 days after eating contaminated products; include abdominal pain, headache, nausea, vomiting, diarrhea for 3-7 days

• Reservoir - intestinal tracts of chickens, turtles

• Infection - ingestion of fecal-contaminated food or water. Poultry products most common source in USA (e.g., undercooked chicken, raw eggs, potato salad).

• Common at community picnics if food not refrigerated properly “food poisoning”
Epidemiology of Nontyphoidal Salmonellae

In 1999, incidence rate of salmonellosis was highest among nine food-borne diseases under Active surveillance.

1.4 million cases annually

Salmonellosis associated with exotic pets is a resurgent public health problem—est. 3-5% of cases associated with exposure to exotic pet, esp. reptiles

Antimicrobial resistance increasing worldwide—consequence of widespread use of antimicrobials for empiric treatment of febrile syndromes and as growth promoters in animal production.

- Recent emergence of S. typhimurium strain (DT104) with multi-drug resistance—assoc. with greater morbidity & mortality
The Salmonella Target the Peyer’s Patch of the Terminal Ileum

This is the target as well for a number of enteric pathogens including *Yersinia enterocolitica*, *EHEC*, *EPEC*, *Listeria monocytogenes* and even *Reovirus*
PATHOGENESIS OF SALMONELLA

GASTROENTERITIS

Stomach acid
Bile

Entry and survival in spacious phagosomes
Recruitment of inflammatory cells
Induction of apoptosis

To spleen and liver

TYPHOID
The surface of the Dome of the Peyer’s Patch appears “Moth Eaten”. Actually there are two kinds of cells. The enterocytes with a uniform surface of microvilli and the other, M-cells, seen here in outline as being covered with a less pronounced number of shorter microvilli.
Transmission Electron Micrograph of an M cell. Note the thin band of cytoplasm separating the lumen of the bowel from the Antigen presenting cells L-lymphocytes and Ma, macrophages. The arrow points to a bacterium being endocytosed.
Surface of Peyer’s patch showing the outline of M cells

Entry of Salmonella into M cells showing marked cytoskeletal rearrangement
This transmission electron micrograph shows Salmonella entering into an M cell. The entering bacteria mediate a marked cytoskeletal re-arrangement. Note that the adjacent enterocytes remain uninfected. The cells immediately beneath the M cell are part of the germinal center composed of elements of the immune system.
The Capacity of Salmonella to Enter into Cells is Associated with an Inherited Pathogenicity Island
Spi-1 maps at centisome 63 on the S. typhimurium chromosome and encodes genes associated with the ability of Salmonella to invade eucaryotic cells. Spi-1 encodes a Type III secretion apparatus designed to facilitate controlled transfer of virulence associated effector proteins into targeted host cells.
Salmonella Is Cytotoxic for Macrophages

Invasion → “splashes” and death

TUNEL+

Wild-type

Non-invasive SPI 1 mutant

SPI 1= Salmonella Pathogenicity Island
Salmonella Induces Rapid Macrophage Death
Caspases Are Required for Apoptosis

denotes cleaves after Asp residues
cysteine protease

Caspase Cascade

Signal X

initiator pro-caspase X

initiator pro-caspase Y

effector pro-caspases

substrates

Death

2 major groups

caspase-1 (ICE)
caspase-4
caspase-5
caspase-11 (murine)
caspase-12 (murine)
caspase-13

Activation of proinflammatory cytokines

Cell Death

caspase-2
caspase-3
caspase-6
caspase-7
caspase-8
caspase-9
caspase-10

Cell Death
Caspase-1 Cleaves pro-IL-1β and pro-IL-18

ProIL-1β (inactive)

Asp-Ala

N

Asp-Ala

C

Active casp-1

ICAM-1, IL-18
(active, secreted)

INFLAMMATION
PMN recruitment
IFN-γ induction

CARD= caspase activation and recruitment domain

Hersh et. al. 1998
Remarkably, the Ability of Salmonella to Replicate Intracellularly and to Cause Systemic Infection is Also Associated With the Inheritance of Several Other Pathogenicity Islands.
<table>
<thead>
<tr>
<th>Chromosomal map position</th>
<th>Island or islet</th>
<th>Virulence phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td><em>ivi</em>VI A, B</td>
<td>adhesion ?</td>
</tr>
<tr>
<td>7</td>
<td><em>pagC-msgA</em></td>
<td>intra-macrophage survival</td>
</tr>
<tr>
<td>25</td>
<td><em>sifA</em> (1.6 kb)</td>
<td>filamentous structures in epithelial cells</td>
</tr>
<tr>
<td>27</td>
<td>SPI-2 (40 kb)</td>
<td>intra-macrophage survival; systemic growth</td>
</tr>
<tr>
<td>30</td>
<td>SPI-1 (40 kb)</td>
<td>epithelial cell invasion</td>
</tr>
<tr>
<td>63</td>
<td>SPI-3 (17 kb)</td>
<td>intra-macrophage survival</td>
</tr>
<tr>
<td>82</td>
<td>Vi (118 kb)</td>
<td>Typhi only capsule production and other unknowns</td>
</tr>
<tr>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
EVOLUTION OF THE SPECIES SALMONELLA

Species | Subspecies | Mainly isolated from
--- | --- | ---
Salmonella enterica | I | Warm-blooded animals
S. enterica | VI | 
S. enterica | II | 
S. enterica | IIIb | Cold-blooded animals
S. enterica | IV | 
S. enterica | VII | 
S. enterica | IIIa | 
Salmonella bongori | V | 
Escherichia coli | | 
Shigella spp. | | 

Time scale: ~120-160 ~80 ~35-40 0 millions of years ago
Table 16–4. Clinical diseases induced by salmonellae.

<table>
<thead>
<tr>
<th></th>
<th>Enteric Fevers</th>
<th>Septicemias</th>
<th>Enterocolitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation period</td>
<td>7–20 days</td>
<td>Variable</td>
<td>8–48 hours</td>
</tr>
<tr>
<td>Onset</td>
<td>Insidious</td>
<td>Abrupt</td>
<td>Abrupt</td>
</tr>
<tr>
<td>Fever</td>
<td>Gradual, then high plateau, with “typhoidal” state</td>
<td>Rapid rise, then spiking “septic” temperature</td>
<td>Usually low</td>
</tr>
<tr>
<td>Duration of disease</td>
<td>Several weeks</td>
<td>Variable</td>
<td>2–5 days</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>Often early constipation; later, bloody diarrhea</td>
<td>Often none</td>
<td>Nausea, vomiting, diarrhea at onset</td>
</tr>
<tr>
<td>Blood cultures</td>
<td>Positive in 1st–2nd weeks of disease</td>
<td>Positive during high fever</td>
<td>Negative</td>
</tr>
<tr>
<td>Stool cultures</td>
<td>Positive from 2nd week on; negative earlier in disease</td>
<td>Infrequently positive</td>
<td>Positive soon after onset</td>
</tr>
</tbody>
</table>

Prototype  

*S. typhi*  
*S. cholerasuis*  
*S. typhimurium*
Salmonella Enteric Fever (Typhoid Fever)

• Symptoms: abdominal pain, weakness, fever, enlarged spleen, (diarrhea not prominent). "Rose Spots" (faint rash) on abdomen and chest (easily overlooked) where organisms multiplying.
• Typhoid Fever is common outside industrialized nations; seen in travelers to/from endemic areas (e.g., Mexico, Latin America, Asia, India). ~500 cases/yr in the USA
• Infectious Dose - High: $10^5$ organisms for an ID$_{50}$

Frequency distribution of the incubation periods in non-immune subjects infected with $10^5$ S. Typhi (Quailles strain) given in milk [taken from Naylor, 1983]
<table>
<thead>
<tr>
<th>Conditions That Predispose to <em>Salmonella</em> Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased gastric acidity (e.g., secondary to antacids, H₂ blockers, or gastric resection)</td>
</tr>
<tr>
<td>Decreased gastrointestinal motility (e.g., secondary to opiates)</td>
</tr>
<tr>
<td>Alterations of normal intestinal flora (e.g., secondary to broad-spectrum antibiotics, purgatives, or bowel surgery)</td>
</tr>
<tr>
<td>Acquired immunodeficiency syndrome</td>
</tr>
<tr>
<td>Lymphoproliferative disease</td>
</tr>
<tr>
<td>Conditions associated with &quot;macrophage blockade&quot; (hemolysis, bartonellosis, malaria, histoplasmosis)</td>
</tr>
<tr>
<td>Inflammatory bowel disease (possible predisposing condition)</td>
</tr>
<tr>
<td>Schistosomiasis</td>
</tr>
</tbody>
</table>
S. typhi Epidemiology

- 12 to 33 million cases per year

- Endemic in Indian subcontinent, Southeast Asia, S. & C. America, and Africa

- Recent outbreaks in eastern Europe following political and social collapse

- Disease much more severe in children <1 yr, although highest incidence occurs in children 1 to 12 years of age

- Antimicrobial-resistant strains -- use of “over-the-counter” antibiotics
The Pathogenesis of Typhoid

**Fig. 13.5** The pathogenesis of typhoid fever.
Rose spots of typhoid fever
Bone marrow biopsy of patient with typhoid fever
Specimen of liver parenchyma from a Patient with Typhoid Fever showing mild Cellularity of the Portal Tract (P) and a Typhoid Nodule (Arrow) within the lobule.
Hepatic lobule from a patient with Typhoid Fever showing a typhoid nodule composed of hyperplastic Kupffer’s cells and lymphocytes.
Cholecystitis and Cholelithiasis following Typhoid fever
Ulcerated Peyer’s Patches of Typhoid Fever
S. typhi

M cell

Tight junction

Macrophage

First exposure of Peyer’s patch to S. typhi

Mesenteric lymph node

Blood

Blood

Liver

Spleen

Peyer’s patch ulcer

S. typhi re-enters GI tract

2nd exposure of Peyer’s patch to S. typhi

Key:

Peyer’s patch

Salmonella typhi

Red blood cells

Necrotic Peyer’s patch

Macrophage

Lymph node

T cells

TRENDS in Microbiology
Cholera Vibrios
John Snow was a physician working in London during an outbreak of cholera in the 18th Century. He noted the houses where individual cases of cholera clustered in a small block of streets near Piccadilly in central London. He further noted that the cases had all used a water pump in Broad Street. Previously, cholera was believed to be spread by “bad air”. He fought to have the handle removed from the broad Street water pump and was eventually successful. This stopped the local outbreak dramatically and was perhaps the first use Epidemiology to control a contagious disease.

ABOVE: Part of John Snow’s map of Soho, showing the position of the pump in Broad Street. The small lines within each block of buildings represent the number of fatal cases in each house. The Wellcome Institute Library

John Snow’s Discovery of the “Broad Street Pump”
*Vibrio cholerae* on 5% sheep blood agar (left). Colonies of *V. cholerae* grow well on most laboratory media. Growth is enhanced by the addition of 1% salt and it tolerates 3%.

*V. cholerae* on thiosulfate-citrate-bile slats sucrose (TCBS) agar (right). Colonies appear yellow due to sucrose fermentation. Most other gram negative bacteria are inhibited in their growth on this medium.
Recorded Pandemics of Cholera

- **I. Classic (Pandemics 1–6: 1817–1923)**
- **II. El Tor (7th Pandemic: 1961–present)**
- **III. O139-Bengal (8th Pandemic: 1992–present)**

Key locations and years of the pandemics are marked on the map.
THE PATHOGENESIS OF CHOLERA

V. cholerae ingested in large numbers

- sensitive to stomach acid
- large dose needed to cause disease unless patient achlorhydric or taking antacids

- colonization of small intestine depends on motility (polar flagella)
- production of mucinase
- attachment to specific receptors

massive loss of fluid and electrolytes
- (no damage to enterocytes; no blood or WBC in stool)

- toxin production
ROLE OF ENTEROTOXIN. EFFECTS OF FEEDING PURE TOXIN TO VOLUNTEERS ORALLY.

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Incubation Time</th>
<th>Stools Produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 ug</td>
<td>NO EFFECT</td>
<td></td>
</tr>
<tr>
<td>5.0 ug</td>
<td>8.8h incubation</td>
<td>2.5l -&gt; 12.3l</td>
</tr>
<tr>
<td>25 ug</td>
<td>6.0h</td>
<td>21.9l -&gt; 48.5l</td>
</tr>
</tbody>
</table>
The “rice-water” stool of cholera
The Cholera Cot
Stop Trot

<table>
<thead>
<tr>
<th>Oral rehydration salt solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bicarbonate solution</strong></td>
</tr>
<tr>
<td>Dissolve in 1 liter of potable water:</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Sodium bicarbonate (Sodium hydrogen carbonate)</td>
</tr>
<tr>
<td>Potassium chloride</td>
</tr>
<tr>
<td>Glucose, anhydrous</td>
</tr>
</tbody>
</table>

| **Citrate solution**          |
| Dissolve in 1 liter of potable water: | grams |
| Sodium chloride               | 3.5   |
| Trisodium citrate, dihydrate  | 2.9   |
| Potassium chloride            | 1.5   |
| Glucose, anhydrous            | 20.0  |
Vibrio cholera Virulence Factors

Polar flagellum

Cholera toxin

Tcp Pili

Colonization of Intestinal Mucosa

Other toxins ie Zot, Ace?

Capsule (O139 strains)?
Evolution of Pathogenic *V. cholerae* Strains

\[ \text{Tcp PAI} \rightarrow \text{Tcp Pili (serve as a Receptor For CTX phage)} \rightarrow \text{CTX phage} \rightarrow \text{Cholera toxin} \]

**V. cholerae** (ancestral strain)

**Tcp** = toxin coregulated pili
Historically enterotoxigenic *V. cholerae* had fallen into serotype O1 until about 20 years ago. Then an outbreak of cholera-like disease was associated with a *V. cholerae* O139 in Bangladesh. This *V. cholerae* derivative was found to cause a significant level of disease over the next decade. Examination of the chromosome of these isolates showed they had acquired a new block of genes for LPS biosynthesis. This variant may have been a result of immune selection against LPS as anti-O antibodies are known to be vibriocidal.
Campylobacter jejuni

- Motile, comma-shaped gram negative rods (closely related to Helicobacter)
- Grow best in microaerophilic environment
- Grows best at 42°C and require special media for isolation
- Higher incidence in developing countries
- ~2.4 million infections annually in U.S.
Modes of *C. jejuni* transmission to humans

- Contaminated poultry
  - 50-98% of commercial poultry products contain live *C. jejuni*
  - *C. jejuni* is a commensal in birds and many other animals
- Raw milk
- Contaminated water supplies
- Household pets
  - Campylobacter enteritis is truly a zoonosis
Campylobacter jejuni affects more people than Salmonella and Shigella combined.

![Graph showing cases reported in study by month for Campylobacter, Salmonella, Shigella, and E. coli 0157 over the year 1996.]

CDC FoodNet (1997) Annual Report
C. jejuni causes severe gastroenteritis and inflammation of the large bowel

Uninfected normal colon

Two days post-infection

Gram stain of a stool sample showing leukocytes (PMN) and slightly curved, comma shaped, gull-wing appearing bacteria typical of *Campylobacter jejuni*
C. Jejunii clinical manifestations

- Acute enteritis most common (incubation period 18 h to 8 d)
- Prodrome of fever, headache, myalgia and malaise 12-24 hr prior to GI symptoms
- Most common symptoms are diarrhea, malaise, fever and abdominal pain
- Diarrhea may range from massive watery to grossly bloody stools. Tenesmus is common.
- May last 1 week or longer

Clinically very similar to Salmonella and Shigella
  → increased severity of abdominal pain
  → higher proportions w/ fever (63%)
  → vomiting (28%)
  → blood in stools (31%)
C. Jejunii clinical manifestations (cont.)

- **Sequelae**
  - Inflammatory Bowel Disease
    - Reactive arthritis
    - Guillain-Barré Syndrome (.1% of cases)
      - acute ascending bilateral paralysis
      - →→ May be due to molecular mimicry (LOS and Ag-crossreactivity w/ sialylated host glycolipids i.e. GM1 ganglioside
Pathogenesis of *C. jejuni* infection

**Intimate host cell-pathogen interaction**

- adhesion
- invasion
- translocation
- intracellular survival

**Known *C. jejuni* virulence factors**

- flagella, capsule, LOS
- cytolethal distending toxin (CDT)
- pVIR: type IV secretion system

Colonization factors for chickens

- Secreted factors (ciaB, pldA)
- Adhesins (cadF, peb1A, jlpA)
- Stress response proteins (dnaJ, sodB)
- Motility (cheY, flaA)
- Signal transduction (racRS)
Helicobacter pylori

- Helical, gram negative bacterium
- Microaerophilic
- Acid-tolerant
- Polar, sheathed flagella
## History of the discovery of *Helicobacter pylori*
(adapted from “*Helicobacter Pioneers*”)

<table>
<thead>
<tr>
<th>Year</th>
<th>Author(s)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1875</td>
<td>Bottchet/Letulle</td>
<td>Bacteria in ulcer margin</td>
</tr>
<tr>
<td>1892</td>
<td>Bizzozero</td>
<td>Described for first time presence of helicobacters in stomach of mammal (dog)</td>
</tr>
<tr>
<td>1906</td>
<td>Krienitz</td>
<td>Spirochetes in stomach w/ gastric cancer</td>
</tr>
<tr>
<td>1954</td>
<td>Palmer</td>
<td>No spirochetes detected using H&amp;E in 1140 suction biopsies →→ no attention for next 20 years</td>
</tr>
<tr>
<td>1975</td>
<td>Steer</td>
<td>Used EM and found bact. close to surface of gastric epithelium</td>
</tr>
</tbody>
</table>
1979 Warren Spiral bact. in the human stomach; used Warthin-Starry silver stain

1983 Warren *Helicobacter* present in patients w/ active gastritis, duodenal ulcer, gastric ulcer

1983 Marshall *H. pylori* isolated and cultured (Koch’s 2nd postulate)

1985-87 Marshall inoculation of himself w/ *H. pylori* (Koch’s 3rd postulate)

**Koch’s Postulates to prove Disease is Caused by Microbe:**

First postulate- microbe must be associated with the lesions of the disease

Second postulate- microbe must be isolated from the lesions of the disease as a pure culture

Third postulate- the pure culture of the microbe must cause the symptoms of the disease if it is inoculated into humans or animals

Fourth postulate- the microbe must be reisolated in pure culture from subjects inoculated for third postulate
The landscape
Gastric pit
Mucous layer, pH 7 to 8
Lumen of stomach, pH 1 to 2
Tests for presence of ammonia

CLOtest™

Detected the urease enzyme of
Helicobacter pylori

Positive

Negative

Manufactured by Delta West Pty Ltd
15 Brodie Hall Drive
Bentley 6102
Western Australia

U.S. Patent No. 4 748 113
U.S. Distributor Tri-Med Specialties Inc
9531 Alden, Lenexa KS 66215
Detection methods for H. pylori

Noninvasive detection methods
- Breath test
  - Rapid presumptive diagnosis
- Serology
  - Definitive diagnosis

Invasive detection methods (biopsy)
- Urease test
  - Stain of histologic section
  - Culture
    - Colonies are:
      - Small
      - Mucoid
    - Biochemical analysis:
      - Oxidase
      - Catalase
      - Urease
  - Gram
  - Warthin-Starry
  - Giemsa
  - Acridine orange
  - Rapid presumptive diagnosis
  - Definitive diagnosis
Prevalence of H. pylori infection correlates best socio-economic status rather than race. In the United States, probability of being infected is greater for older persons (>50 years = >50%), minorities (African Americans 40-50%) and immigrants from developing countries (Latino > 60%, Eastern Europeans > 50%). The infection is less common in more affluent Caucasians (< 40 years = 20%).
Diseases Caused by Helicobacter pylori

- Nonatrophic pangastritis
- Normal stomach
- Chronic gastritis

- Duodenal ulcer
- Gastric ulcer
- MALT lymphoma
- Gastric cancer
Persons in the infected group develop duodenal ulcer at the rate of about 1% per annum so that approximately one third eventually have peptic ulcer disease. The smaller circles represent diseases associated with H. pylori.

Nearly all persons with duodenal ulcer are infected.

Gastric ulcer is usually caused by H. pylori, but about 30% of gastric ulcers in the United States occur in persons without H. pylori and can be related to aspirin and other non steroidal anti-inflammatory drugs.

Most gastric adenocarcinomas and lymphomas occur in persons with current or past infection with H. pylori. In developing countries the ulcer groups are smaller and the gastric cancer group may be larger. For example, in northern Brazil, gastric cancer is the most common malignancy in men.
39 year-old woman with unexplained anemia and occult gastrointestinal bleeding. Endoscopy revealed **erosive antral gastritis**; biopsies contained *Helicobacter*

44 year-old man with abdominal pain and occult gastrointestinal tract bleeding. Endoscopy revealed **diffuse gastritis** involving and distal gastric body and antrum. Clotest was positive for *Helicobacter*

72 year-old woman with hematemesis (vomiting blood). In addition to this inflammatory process involving the gastric body and antrum, she also had a small **Gastric ulcer**. Biopsies and Clotest were both positive for *Helicobacter pylori*.
72 year-old man who presented with acute hematemesis. Endoscopy revealed a small bleeding ulcer (left and center); bleeding was massive despite the small size of the ulcer. Bleeding was controlled by banding (right). The blue rubber band and the mucosal bleb created by banding are visible through the translucent endoscope attachment used in the banding process.

77 year-old man presented with acute hematemesis (vomiting blood). Exam revealed this ulcer (at 9:00 o'clock position) with a white, fibrinous base, and a dark, protruding visible vessel, signifying the site of recent bleeding. Biopsies were positive for Helicobacter.
Uninfected Mouse stomach

H. pylori Infected, Mouse Stomach (C57BL/6)
*H. pylori*-infected mouse stomach, Arrow indicates a Lymphoid Aggregate
Helicobacter pylori infection – MALT
Gastric Cancer
Natural History of *Helicobacter pylori* Infection

- **High level of acid production**
  - Normal gastric mucosa → Acute *H. pylori* infection → Chronic *H. pylori* infection → Antral-predominant gastritis → Duodenal ulcer
  - Nonatrophic gastritis
  - Corpus-predominant atrophic gastritis → Gastric ulcer → Intestinal metaplasia → Dysplasia → Gastric cancer

- **Low level of acid production**
  - Asymptomatic *H. pylori* infection

Timeline:

- Childhood
- Advanced age
Treatment of H. pylori Infection

★ Triple Therapies

- proton-pump-inhibitor + amoxicillin + clarithromycin

- Taken twice a day for 2 weeks

★ Eradication of bacteria and reversal of disease (if not too far along)
*H. pylori* Virulence Determinants

- Adhesins
- Flagella
- Urease
- Iron acquisition
- Neutrophil activating protein (*napA*)
- CagA
- Vacuolating cytotoxin (*vacA*)
Two Types of \textit{H. pylori}

- One Type Produces the CagA Antigen
- Both CagA+ & CagA- Strains Cause Gastritis
- Cag A+ Associated with Ulcers & Other Severe Disease
- Cag A + Often Induce:
  - IL-8 secretion
  - NF-kB activation
  - Actin cytoskeleton rearrangement
Risk for gastric cancer in CagA+ and CagA- subjects compared to uninfected subjects

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>Adjusted OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CagA+</td>
<td>66/80</td>
<td>49/105</td>
<td>6.2</td>
<td>2.9 - 13.3</td>
</tr>
<tr>
<td></td>
<td>(83%)</td>
<td>(47%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CagA-</td>
<td>19/33</td>
<td>41/97</td>
<td>2.0</td>
<td>0.8 - 4.6</td>
</tr>
<tr>
<td></td>
<td>(58%)</td>
<td>(42%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Adjusted for age, sex, race
**H. pylori : CagA**

- Immunodominant protein 120-130 kDa
- 60% of patients in USA and Europe produce antibodies to CagA
- 90% of strains from patients with ulcers are CagA
- Disruption of the CagA gene does not influence Urease or VacA production
- Initially, no known function
PAI G+C content differs from overall genome G+C content: 35 mol% vs genome 39 mol%.

Flanking direct 31 bps repeats of the glutamate racemase gene at the integration site.

**H. pylori strain 26695 genome (1,667,867 bp)**

**cag** pathogenicity island (37,000 bp)

The proteins encoded by these genes assemble to form a complex type IV secretion apparatus capable of delivering CagA from the bacterium into host cells.

Translocation of CagA into gastric epithelial cells

Phosphorylation of CagA by host-cell kinases c-Src and Lyn

Binding to and activation of cellular phosphatase SHP-2

Growth factor–like response in host cell, cytoskeletal rearrangements
H. pylori Induces Morphological Changes in Gastric Epithelial Cells

Segal et al, PNAS(1999), 96(25)
Time-lapse Video Microscopy of *H. pylori*-induced Elongation of Host Cells

Uninfected cells

Infected with *H. pylori*

😊 From Manuel Amieva
Dramatic Morphological Changes are Dependent on CagA Delivery

Infected w/ wild-type CagA+ Infected w/ mutant CagA-
EPIYA motifs (4 in carboxy terminus of G27 CagA) are Y phosphorylated by c-Src and Lyn in AGS cells.

pY residues of EPIYA bind to SH2 domains of the tyrosine phosphatase SH2P which leads to changes in cell shape in AGS cells.
H. pylori in a biopsy of pre-pyloric mucosa
In Well Developed Polarized Monolayers
*H. Pylori* Co-localizes With the Tight Junctions

😊 From Manuel Amieva
*H. Pylori* causes abnormal distribution of TJ protein ZO-1 and bugs co-localize to these areas
**Barrier function**

**Generation and Control of Polarity during wound healing and development.**

**Regulation of Cell Proliferation**
Host-Pathogen Interactions

- host response large role in damage to gastric epith.
  - chemokines
  - cytokines
  - chronic inflammation
  - Th1 response
Microbial Humor
Revisiting Shigellosis and a Look at Listeria monocytogenes

The Parallel Basis of the Bacterial Strategy to be Facultative Intracellular Parasites—and Viruses Too
The “classic” dysentery stool showing blood, mucus and small volume. Microscopically, there are sheets of leukocytes.
Histopathology of Shigellosis showing severe inflammation and blunting of the villi.
### Intracellular bacteria that induce and/or inhibit host cell apoptosis

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Host cells</th>
<th>Apoptosis</th>
<th>Host targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shigella (IpaB)</td>
<td>Macrophages</td>
<td>Induction</td>
<td>Caspase-1</td>
</tr>
<tr>
<td>Salmonella (SipB)</td>
<td>Macrophages</td>
<td>Induction</td>
<td>Caspase-1</td>
</tr>
<tr>
<td>Listeria (LlyO)</td>
<td>Hepatocytes, Lymphocytes, Dendritic</td>
<td>Induction</td>
<td>Unknown</td>
</tr>
<tr>
<td>Legionella</td>
<td>Macrophages, Epithelial</td>
<td>Induction</td>
<td>Caspase-3</td>
</tr>
<tr>
<td>Yersinia (YopJ/YopP)</td>
<td>Macrophages</td>
<td>Induction</td>
<td>NF-B, TNF, MAPKK</td>
</tr>
<tr>
<td>Mycobacterium</td>
<td>Macrophages</td>
<td>Induction</td>
<td>TNF-, caspase-1</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>Epithelial cells, Macrophages</td>
<td>Induction inhibition</td>
<td>Caspase-3, cyt c</td>
</tr>
<tr>
<td>Rickettsia</td>
<td>Epithelial cells</td>
<td>Induction</td>
<td>NF-B</td>
</tr>
<tr>
<td>Coxiella</td>
<td>Macrophages</td>
<td>Induction</td>
<td>TNF-</td>
</tr>
</tbody>
</table>

[a] Abbreviations: cyt c, cytochrome c; MAPKK, mitogen-activated protein kinase kinase; NF-B, nuclear factor B; TLR-2, toll-like receptor; TNF-, tumor necrosis factor.
*Shigella flexneri* induced macrophage apoptosis initiates inflammation. (a) *Shigella flexneri* crosses the colonic epithelium by subverting the ability of M cells to translocate luminal antigens. (b) In the lamina propria, *S. flexneri* is phagocytosed by resident tissue macrophages in close proximity to the M cells. (c) After phagocytosis the bacteria escape from the phagolysosome and gain access to the cytoplasm of the macrophage. (d) *S. flexneri* secrete IpaB which directly interacts with ICE leading to cleavage of immature IL-1β to produce the biologically active, mature form of the cytokine. Concomitantly, the macrophage undergoes apoptosis and IL-1β is released into the lamina propria. (e) IL-1β acts as a potent chemotactic stimulus for the infiltration of neutrophils into the inflamed lamina propria. The neutrophils enhance inflammation by releasing proinflammatory molecules and disrupt the integrity of the epithelial barrier through transmigration into the colonic lumen.
Yersinia being endocytosed into M-cell
<table>
<thead>
<tr>
<th>CLINICAL MANIFESTATION</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute diarrhea</td>
<td>Children younger than 5 years</td>
</tr>
<tr>
<td>Mesenteric adenitis and terminal ileitis</td>
<td>Children older than 5 years and adults</td>
</tr>
<tr>
<td>Polyarticular nonsuppurative arthritis, ankylosing spondylitis, Reiter syndrome</td>
<td>HLA-B27-positive persons following <em>Y. enterocolitica</em> infection</td>
</tr>
<tr>
<td>Erythema nodosum</td>
<td>Predominantly in women</td>
</tr>
<tr>
<td>Septicemia</td>
<td>Persons with underlying diseases</td>
</tr>
<tr>
<td>Metastatic infections</td>
<td>Following septicemia</td>
</tr>
</tbody>
</table>
*Shigella flexneri* induced macrophage apoptosis initiates inflammation. (a) *Shigella flexneri* crosses the colonic epithelium by subverting the ability of M cells to translocate luminal antigens. (b) In the lamina propria, *S. flexneri* is phagocyted by resident tissue macrophages in close proximity to the M cells. (c) After phagocytosis the bacteria escape from the phagolysosome and gain access to the cytoplasm of the macrophage. (d) *S. flexneri* secrete IpaB which directly interacts with ICE leading to cleavage of immature IL-1β to produce the biologically active, mature form of the cytokine. Concomitantly, the macrophage undergoes apoptosis and IL-1β is released into the lamina propria. (e) IL-1β acts as a potent chemotactic stimulus for the infiltration of neutrophils into the inflamed lamina propria. The neutrophils enhance inflammation by releasing proinflammatory molecules and disrupt the integrity of the epithelial barrier through transmigration into the colonic lumen.
## Appendix 20-1. General Characteristics of Some Enterobacteriaceae

<table>
<thead>
<tr>
<th>Organism</th>
<th>Serologic Type(s) (Antigens)</th>
<th>Lactose</th>
<th>Indole</th>
<th>Urease</th>
<th>Hydrogen Sulfide</th>
<th>Motility</th>
<th>Other</th>
<th>Major Disease(s)</th>
<th>Found in Normal Flora</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>150+ (O, K, H)</td>
<td>+</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td>Urinary tract infections; diarrhea; opportunistic</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>10 (O)</td>
<td>-</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Dysentery (type 1, severe)</td>
<td>No</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>6 (O)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Dysentery</td>
<td>No</td>
</tr>
<tr>
<td><em>Shigella boydii</em></td>
<td>15 (O)</td>
<td>-</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Dysentery</td>
<td>No</td>
</tr>
<tr>
<td><em>Shigella sonnei</em></td>
<td>1 (O)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Dysentery</td>
<td>No</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>72 (K)</td>
<td>+</td>
<td>-</td>
<td>+*</td>
<td>-</td>
<td>-</td>
<td>Encapsulated</td>
<td>Pneumonia; opportunistic</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Enterobacter sp.</em></td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td>Opportunistic</td>
<td>No</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td></td>
<td>-</td>
<td>-</td>
<td>v*</td>
<td>-</td>
<td>+</td>
<td>Red pigment</td>
<td>Opportunistic</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Salmonella serotypes</em></td>
<td>1500+ (O, H)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Diarrhea</td>
<td>No</td>
</tr>
<tr>
<td><em>Salmonella choleraesuis</em></td>
<td>1 (O, H)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>v</td>
<td>+</td>
<td></td>
<td>Bacteremia</td>
<td>No</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>1 (O, H, K)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Enteric (typhoid) fever</td>
<td>No</td>
</tr>
<tr>
<td><em>Salmonella paratyphi A.</em></td>
<td>(O, H)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Diarrhea; enteric fever</td>
<td>No</td>
</tr>
</tbody>
</table>
Development of Acquired Immunity in Typhoid
A large number of differential media have been developed for use in the clinical laboratory. One of these, Hektoen agar is illustrated here. Lactose, sucrose and salicin-fermenting organisms appear yellow, non-fermenters appear colorless and $\text{H}_2\text{S}$ production turns the colonies black.
IL-1β is processed to its mature form in Salmonella-infected macrophages.

Caspase-1 (ICE) participates in the processing of pro-IL-1β and pro-IL-18 to their mature forms, leading to inflammation and apoptosis.

PMNs (polymorphonuclear leukocytes) are involved in the inflammatory response.
Infectious Causes of Human Malignancy: Bacteria and Parasites

- Schistosomiasis, Bladder and Colon Cancer
- Liver Flukes and Biliary Cancer
- Draining Osteomyelitis and Skin Cancer
- S. typhi/paratyphi and Gall Bladder Cancer
- Perhaps Mycoplasma and Lung Cancer, M. ulcerans and Skin Cancer as well as Bacterial Infection and Colon Cancer
- Helicobacter pylori and Gastric Adenocarcinoma
<table>
<thead>
<tr>
<th>Preformed Toxin</th>
<th>Toxin Production In Vivo</th>
<th>Tissue Invasion</th>
<th>Toxin Production and/or Tissue Invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>C. perfringens</td>
<td>C. jejuni</td>
<td>V. parahaemolyticus</td>
</tr>
<tr>
<td>B. cereus</td>
<td>B. cereus (long incubation)</td>
<td>Salmonella</td>
<td>Y. enterocolitica</td>
</tr>
<tr>
<td>C. botulinum</td>
<td>C. botulinum (infant botulism)</td>
<td>Enterotoxigenic E. coli</td>
<td>Invasive E. coli</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>V. cholerae O1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>V. cholerae non-O1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Verotoxigenic E. coli</td>
</tr>
<tr>
<td>INCUBATION PERIOD</td>
<td>VOMITING</td>
<td>ABDOMINAL CRAMPS</td>
<td>FEVER</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------</td>
<td>------------------</td>
<td>-------</td>
</tr>
<tr>
<td>1–6 hr</td>
<td>++</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>8–16 hr</td>
<td>±</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>12–72 hr</td>
<td>±</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>12–72 hr</td>
<td>++</td>
<td>+</td>
<td>±</td>
</tr>
</tbody>
</table>
Food Poisoning Outbreak

In February 1975 a fully loaded 747 left Anchorage, Alaska for Copenhagen, Denmark. Just before landing, 196 (56%) of the 343 passengers and one crew member developed

- Diarrhea - 88%
- Vomiting - 82%
- Abdominal pain - 74%
- Fever - 1%

143 passengers and the crew member were hospitalized. 30 required IV fluids. All recovered rapidly within 24 hr.

What are the possible agents?
A snack was served shortly before takeoff, and breakfast 2 hours before arrival. The cook who had sliced the ham for omelettes had an inflamed skin lesion of the right hand. The ham was placed on the omelettes which were left at room temperature for 6 hours in the galley unrefrigerated until just before breakfast when they were heated and served.

What is your diagnosis now?
A snack was served shortly before takeoff, and breakfast 2 hours before arrival. The cook who had sliced the ham for omlettes had an inflamed skin lesion of the right hand. The ham was placed on the omlettes which were left at room temperature for 6 hours in the galley unrefrigerated until just before breakfast when they were heated and served.

Staphylococcus aureus was isolated from the finger of the cook and the same strain was found in the omelette.
Food poisoning outbreak

300 students at a residential college developed diarrhea (82%), abdominal cramps (75%), headache and nausea.

Outbreak started in the late afternoon of January 17 and lasted for 30 hours.

Associated with eating lamb stew for lunch on January 17.

Investigation revealed that the lamb was roasted January 16 and served for dinner. Meat was kept for an uncertain time at room temperature. Refrigerated at 47 F.

Meat was removed from refrigerator the next morning, cut into pieces and added to vegetables which had been cooked in a cauldron for 15 min. Stew dipped from cauldron into bowls for serving at lunchtime.

What is your diagnosis?
Food poisoning outbreak

300 students at a residential college developed diarrhea (82%), abdominal cramps (75%), headache and nausea.

Outbreak started in the late afternoon of January 17 and lasted for 30 hours.

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Meat was removed from refrigerator the next morning, cut into pieces and added to vegetables which had been cooked in a cauldron for 15 min. Stew dipped from cauldron into bowls for serving at lunchtime.

C. perfringens isolated from stew.
Food Poisoning Outbreak

A political banquet in Kansas attended by 1300 people was the scene of a food poisoning outbreak. Within 36 hours symptoms began to appear and finally about 900 people became ill with gastroenteritis. 12 people were hospitalized; there were no deaths.

The symptoms were:

- Diarrhea  88%
- Cramps    41%
- Fever     39%
- Vomiting  14%

The median time to onset was 52 hours.

What are the possible agents?
The vehicle of infection appeared to be turkey. A gram negative rod was recovered from the serving table 9 days after the banquet. The banquet refrigerating facilities were found to be defective. The Gram negative organism was subsequently identified as *Salmonella typhimurium*. 
Noninflammatory
(No fecal leukocytes)
Exclude Vibrio (cholerae et al.)
E. coli (LT, ST)
C. perfringens
S. aureus
B. cereus
Giardia, Cryptosporidium
Rotavirus, Norwalk virus

Continue symptomatic therapy
Consider further evaluation
if no resolution

Inflammatory
(fecal leukocytes or lactoferrin or continued illness)
Exclude Shigella
Salmonella
C. jejuni
E. coli (EIEC)
Cytotoxic C. difficile
E. histolytica

Culture for
Shigella
Salmonella
C. jejuni

Consider
C. difficile
Cytotoxin

Consider
Empiric antimicrobial therapy

Parasites
Wet mount
Giardia lamblia
E. histolytica
Concentration
Strongyloides
Acid fast
Cryptosporidium
Isospora belli
Cyclospora
Special trichrome
Microsporidium

Specific antiparasitic therapy
Moderate Dehydration
Up to 10% body weight lost
Pallor is striking
Initial Irritability gives way to listlessness
Sunken eyes from loss of water from retro-orbital fat pad

Loss of skin turgor and Elasticity
Comparison of the epidemiology of *C. jejuni* (---) and *H. pylori* (----) by seasonal distribution by month (panel A) and by age (panel B) in the United States.