Intestinal Disease Pathogenesis:
*Invading and defending the castle*
Intestinal Disease Pathogenesis:

“Bacteria devise a plan to invade the castle”
Pathogenesis: 
*Invading and defending the castle*

Enteric *E. coli, E. coli* Shigella, ExPEC, *Salmonella* (and others)

- How far do the invaders get into the castle? Will they capture the castle?
- It’s a battle between the invaders and the defenders of the castle.
- **Enteric *E. coli* (ETEC, AEEC, STEC)** – swim the moat, set up camp around the outer castle walls, hurl insults and missiles, occasionally capture the castle
- ***E. coli* Shigella** – breach the outer walls, send out raiding parties, wage small pitched battles with defenders, usually do not make it to inner castle
- **ExPEC, *Salmonella*** – lay siege, infiltrate, & invade deep into castle, wage major battles with defenders, and often capture the castle
Enterobacteriaceae: 
*E. coli* Shigella and *Salmonella*

Course: VPM 201 Fall, 2010
Lecturer: C. Anne Muckle
What is the Bug? *E. coli* Shigella

Former *Shigella* species now called:
- *E. coli* Dysenteriae
- *E. coli* Flexneri
- *E. coli* Sonnei
- *E. coli* Boydii

- Nonmotile and nonlactose fermenters, (which is different from most *E. coli*)

- Diverged from nonpathogenic *E. coli* quite recently (only 35,000 - 270,000 years ago)
- Evolved with humans; very limited host range = humans & nonhuman primates
- Have lost *E. coli* genes and picked up virulence genes by HGT
- Have invasion plasmid (pINV) and PAIs→ invasion proteins, Type III secretion system, enterotoxins
- *E. coli* Dysenteriae 1 acquired *Shiga* toxin gene by a lysogenic phage (phage has integrated in the chromosome)
The *E. coli* Shigella: “Bacillary dysentery” in primates

- Acid resistant; infectious dose is very low (10^-100 organisms)!!
- Cause of bacillary dysentery worldwide, endemic and epidemic
- Young children at greatest risk, a significant cause of death in developing countries
- Fecal-oral transmission, contaminated food and water
- *Cause severe bloody enteritis = dysentery (blood, mucous, pus, PMNs), dehydration, fever*

Fewer than ten ingested bacteria are enough to cause a Shigella infection.
The *E. coli* Shigella: What does the Bug do? Ulcerative colitis

**Bacterial INVASION!**
- *Shigella* bacteria invade **M cells in colon** (M cells cover mucous-associated lymphoid tissue = MALT), kill macrophages, enter enterocytes, spread cell-to-cell in colonic enterocytes, replicates inside enterocytes → tissue destruction & intense inflammatory response by PMNs, macrophages, NKC → **ulcerative colitis**

**Host DEFENSE!**
- PMN response limits invasion by *Shigella* bacteria to the colonic mucosa, necrotic colonic tissue is shed into lumen colon (“casts”)

- Also causes reactive arthritis in people with HLA-B27 HC (B27 gene)
- Only *E. coli* Dysenteriae 1 produces Shiga toxin; causes hemolytic uremic syndrome (HUS) neurological damage, septicaemia, death
E. coli Shigella infection in captive monkeys and apes

- Predisposed by an altered environment with food contaminated by feces on cage floor, stress from crowding, transport, Simian acquired immunodeficiency syndrome (SAIDS).

- Symptomatic and carrier monkeys can infect humans = zoonotic transmission

- Free-ranging gorillas may be infected by humans = reverse (anthropozoonotic) transmission
Salmonella

“Fun with Bacteria” Artwork by Sadie Griffin
Enterobacteriaceae - The Genus *Salmonella*
What is the Bug?

Salmonella nomenclature Trivia:
- There are only **two** species of Salmonella -
  - *Salmonella enterica* and *Salmonella bongori*
- *S. enterica* has six subspecies (groups)
- Salmonella are called serotypes, also called “serovars” (the preferred term); these are designated by an antigenic formula based on serotyping of their O and H antigens
- There are over 2,557 serovars of *S. enterica* (aren’t you thrilled to know all this?)
Q – What Salmonella serovars most commonly cause disease in animals and humans?

*The Group I Salmonella (S. *enterica* subspecies *enterica*) contains the majority of serovars causing disease in animals and humans. (This is only about 50 serovars)*
Enterobacteriaceae - The Genus *Salmonella*

"Naming the Bug" Trivia * (* see disclaimer below)

The Group 1 (*Salmonella S. enterica* subspecies *enterica*) currently has 1,531 serovars

**Only Group I serovars are given a NAME, the rest have antigenic formulas only**

The name usually is the place where that serovar was first isolated, e.g. – Heidelberg, Dublin, Kentucky, Montevideo, Westminster, Johannesburg

BUT there are some older names that do not fit this pattern – e.g. Typhimurium, Enteritidis, Choleraesuis, Bovismorbificans, Gallinarum (Pullorum is an obsolete name; now only called Gallinarum)

Example:

*Salmonella enterica* subspecies *enterica* serovar Typhimurium

It’s antigenic formula is I: 4,5,12:i:1,2

Also just called *Salmonella* Typhimurium for short

The name has a capital first letter and no italics, NOT like the usual format of writing Genus and species names for other bacteria (NOT *S. typhimurium*), because these are not real bacterial species.

*(This is intended as somewhat trivial but hopefully useful information so you won’t phone me someday asking what all these cryptic names and numbers mean on a lab report that you have received.)*
Salmonella characterisation: (More about identifying Salmonella in the Lab)

- **Routine diagnostic labs** can only presumptively identify *Salmonella* by biochemical tests or PCR and by slide agglutination with O-Grouping reagents (e.g. - Poly “O” antiserum)

- We can only report them as “Salmonella species”

- We send cultures to **Salmonella Reference Labs** to have them serotyped, which gives them their serovar formula/name. This confirms their identification.

- Salmonella Reference Labs also phagetype certain *Salmonella* serovars to subtype them e.g. *S. Typhimurium* phagetype (PT) 104, *S. Enteritidis* PT 4

Salmonella Poly O slide agglutination

OIE Reference Laboratory for Salmonellosis, Public Health Agency of Canada, Guelph, Ontario
**Salmonella Umbadah:**
A new serovar, named for the region it was isolated in Sudan

"Nobody knew who I was; I didn't have a name!"

**A Story of Discovery**

Involving cattle, *Salmonella*, researchers & diagnosticians in Sudan, Canada, France, USA, & Germany

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**Salmonella Umbadah: A new *Salmonella* serovar isolated from cattle in Sudan**

H. E. R. El-Hage-El-Chel, M. M. Nor Elmadien, A. A. El Hadidi, M. A. M. Shafig, F. A. Madify, T. C. E. Wilks, M. S. Thiny

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**Keywords** *Salmonella* cattle, *Salmonella* Umbadah, isolation, Sudan

**Introduction**

*Salmonella* is a significant enteric bacterial pathogen affecting human and animal populations worldwide (Dennett et al., 2001). In Sudan, the prevalence of *Salmonella* serovars in cattle is common. Although enteric pathogens are minimally isolated and identified, only a few studies of *Salmonella* infections from cattle in Sudan have been published. Recently, Khalil and colleagues (1993, 1994) identified *Salmonella* serovars from cattle in Sudan. Khalil et al. (2001) isolated and identified *Salmonella* serovar Umbadah from cattle in Sudan. In this study, *Salmonella* serovar Umbadah was isolated from cattle in Sudan.

**Materials and methods**

A total of 150 cattle fecal samples were collected for isolation of *Salmonella* species. *Salmonella* from isolated fecal samples are identified following conventional antigen-hybridization previously described (Amin et al., 2003). *Salmonella* isolates were tested by the disc diffusion method and identified following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 2001). The antimicrobial drug trial was conducted by determining the minimum inhibitory concentrations (MIC) of the antimicrobial agents: amoxicillin, cefotaxime, ceftriaxone, ceftazidime, cotrimoxazole, ciprofloxacin, doxycycline, gentamicin, chloramphenicol, spectinomycin, streptomycin, tetracycline.
**Salmonella identification:**

What do YOU really need to know about this bug to identify it in the Lab?

- **Salmonella** are oxidase negative, gram-negative rods

- They grow at 37°C, but also at 42°C

- *Salmonella* are non-lactose fermenters → colourless colonies on MacConkey agar

- They are motile by flagella, (except Gallinarum)

- *Salmonella* have different biochemical reactions than E. coli – notably, they produce H₂S gas; are indole negative; and have different TSI slant reaction:
  - Salmonella = K/A H₂S +

**H antigen** = flagellar antigen = protein flagellin antigens, gives mobility to *Salmonella* bacteria (they are “swimmers”)

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*Salmonella* on MacConkey agar

*Salmonella* on XLD agar

*Salmonella* on TSI: K/A H₂S +

Positive *Salmonella* motility on MRSV plate

"Fun with Bacteria" Artwork by Sadie Griffin
Salmonella serovars, phagetypes and disease:
Yes, it is important to know the serovar & phagetype of a Salmonella isolate

- Just knowing that a “Salmonella species” has been isolated is really not helpful for disease diagnosis and epidemiological investigations.
- We need to know the STORY:
- Is this a serovar or phagetype commonly seen in this type of animal?
- If not, where did this particular serovar come from?
- Is this a serovar or phagetype known to be multi-drug resistant?
Examples of *Salmonella* serovars and disease: *Salmonella* Stories.....

- A dog with *S*. Infantis from eating pig ear dog treats.

- A child with *S*. Tilene from a pet African pygmy hedgehog.

- A cat with *S*. Typhimurium PT U284 variant. The cat caught and ate a Purple Finch at the backyard bird feeder.

- A child with *S*. Gaminara (pet lizard) *S*. *enterica* ssp. *arizonae* (IIIa): associated with cold-blooded animals, regarded as normal flora. This is why the commercial sale of pet turtles is illegal.

- Sheep and *S*. *enterica* ssp. *diarizonae* (IIIb) 61:k:1,5,(7): considered host-adapted to sheep; incidental, normal flora.

- A dairy farmer with *S*. Meunster from drinking raw milk from the bulk tank.
Many Salmonella serovars have host preferences, some don’t

- Examples of host-adapted Salmonella serovars: These are important causes of septicemia in each particular host
  - Cattle – S. Dublin
  - Pigs – S. Choleraesuis
  - Chickens – S. Gallinarum, S. Heidelberg, S. Enteritidis
  - Humans – S. Typhi and S. Paratyphi
  - Reptiles – Group III

- In contrast, S. Typhimurium and S. Enteritidis have a wide host range

  "We’re so talented, we can infect anybody", we’ve even been called promiscuous!!"

S. Typhimurium is really special as it can infect many host species, but it causes enteritis not septicemia, in contrast to the host-adapted serovars

"I’m really special"
Q. How does the Bug *Salmonella* cause disease?

- *Salmonella* are invasive enteric pathogens and facultative intracellular pathogens (FIPs)
- *Salmonella* can survive a long time in the environment (years!!)
- Animals are infected by ingestion from feed, milk, water, feces

Q. What special virulence factors does this bug have allowing it to invade and survive inside host cells?

- LPS, flagella, fimbriae, serum resistance, antimicrobial drug resistance
- *Salmonella* Pathogenicity Islands (SPI) (five of them) allowing invasion of host (Type III secretory system) and uptake by enterocytes, intracellular survival in macrophages
- Plasmid-encoded virulence factors e.g. - S. Typhimurium 60 Mdal plasmid, S. Enteritidis and 36 Mdal plasmid
Salmonella and INFLAMMATORY DIARRHEAL DISEASE:
(Salmonella can capture the Castle!)

- Salmonella adhere to & invade M cells in Peyer’s patches, reach submucosa & kill macrophages & PMNs, invade & kill enterocytes, reach reticuloendothelial system (RES) = lymph nodes, liver, spleen) causing septicaemia

- Enteritis: bloody diarrhea, necrosis, intestinal casts, anal strictures, dehydration

- Septicemia: endotoxic shock, fever, polyarthritis, osteomyelitis, meningoencephalitis, abortion, death, or recovery with shedding of bacteria from host
Salmonella carriers, shedding, and stress

- *Salmonella* persist in macrophages in lymph nodes & the bile duct, so that recovered animals and humans become carriers and shed bacteria when stressed.

- Stress from crowding, shipping malnutrition, concurrent disease or cancer, treatment with antimicrobials or immunosuppressive drugs

Example - “joy riding of pigs” = shipping stress

How can we reduce stress in the feedlot?
Q. Why is it important to screen large animal patients entering a veterinary teaching hospital for *Salmonella*?

- To check if they are **asymptomatic Salmonella carriers**.

- Example - horse patients in vet hospitals, especially after colic surgery &/or oral antibiotic treatment → *Salmonella* contamination and hospital outbreaks (nosocomial infections).
Salmonella carriers – a human story

*Salmonella* infection can be asymptomatic (carrier animals) or clinical. “Typhoid Mary”, Mary Mallon, a NY domestic cook in early 1900s; was a healthy carrier of typhoid fever & did not wash her hands well enough! She infected many of her clients, more than 50, & three died.
Q. Why don’t standard killed (toxoid) vaccines work very well for Salmonella?

- Killed vaccines are not protective for *Salmonella* as they do not → CMI
- Attenuated live vaccines made with “rough” *Salmonella* mutant strains give a CMI response, without causing lasting infection or severe illness
- Examples of pig vaccines: *Enterisol SC-54*, *(Boehringer Ingelheim)*; and *Argus SC/ST* *(Argus)*, both oral avirulent live *Salmonella* Choleraesuis vaccines
- A new cattle vaccine, *Salmonella* Newport Bacterial Extract, *(Epitopix)*, is a bacterial extract vaccine which stimulates antibodies against the siderophore receptor and porin proteins on *Salmonella* cell wall to block the bacteria’s ability to obtain iron from its environment.
Q. What autoimmune disease can result from *Salmonella* infection in humans?

- As a result of *Salmonella* infection, humans can develop **Postdysenteric Reactive Arthritis** (ReA):
  - During the visit by the Pope John Paul in 1984 to Midland, Ontario, OPP officers got food poisoning with *Salmonella Typhimurium* from eating a boxed lunch. 500 men ate a contaminated roast beef sandwich; 260 were infected; and 19 developed joint disease. ReA is caused by immune-mediated damage to joints, predisposed by possession of the major histocompatibility complex (MHC) gene HLA-B27. In Canada 7% of population carry this gene.
  - ReA is also caused by *Yersinia*, *Shigella*, and *Campylobacter*.
  - *This is an example of autoimmune disease triggered by bacterial infection due to cross-reactivity of antigens.*
Salmonella in the Poultry Industry

*Salmonella* Enteritidis and S. Gallinarum in poultry are reportable diseases in Canada.

*Salmonella* in poultry flocks is endemic because of intensive rearing conditions.

*Salmonella* control procedures (biosecurity, probiotics, serological testing, culling, vaccinations) have not had much impact; the serovars just change over time.

The use of prophylactic use of antibiotics in poultry production selects for resistance in *Salmonella* in chickens, retail chicken meat, and humans (ex. Ceftiofur and S. Heidelberg).

**S. Enteritidis PT 4 (SE PT4)** in poultry causes septicaemia with salpingitis (infection of oviducts) resulting in egg transmission and infection of humans eating raw eggs.

SE PT4 infection is special; the bacteria are inside the egg, not just on outside like other *Salmonella* from fecal contamination of eggs.

Warning: Remember the BRITS who keep their eggs at room temperature.

"Remember, I'm Se PT4, and I am really special, I can infect eggs inside the hen"
Q. Why is salmonellosis important?

- Animals are major source of Salmonella infection in humans = zoonosis

- Salmonellosis is a worldwide public health issue – caused by poor sanitation → contamination of water and food

- Salmonellosis is a major worldwide food safety issue, causing large local and international outbreaks (ex. S. Stanley in alfalfa sprouts, raw almonds, SE PT 4 in eggs), associated with global food marketing

- Salmonellosis in humans is a Reportable Disease in Canada
Prevention of *Salmonella* infections

Remember the risks of pet animals & children, the elderly, immunosuppressed, & AIDS patients; handling calves; pet reptiles; drinking raw milk; eating raw eggs; preparing meals with raw chicken (toasted chicken nuggets are a raw product!); cross-contamination of food in the kitchen (cutting boards, counters, dishcloths)

Cook your chicken well and wash your hands
The role of vets to advise their clients of the risk of Salmonella

The risks of *Salmonella* from raw meat-pet food diets:

See reference: Weese JS, Rousseau J. Survival of *Salmonella* Copenhagen in food bowls following contamination with experimentally inoculated raw meat: Effects of time, cleaning and disinfection. CVJ 2006;47:887-889

- In 2009, the AVC Diagnostic Services Bacteriology Lab isolated *Salmonella* from a dog with diarrhea; the dog was on a raw-food diet.

The risks of *Salmonella*, even in dry pet food diets:

- Listen to AVMA podcast:
- http://www.avmamedia.org/manage/mediaimg/s313-2salmonellapetfoodanimaltracksfinal.mp3

Dr. Scott Weese
A story about raw milk, Schmidt, and Salmonella

Michael Schmidt was acquitted in a Newmarket court this morning of 19 charges of distributing raw milk and raw milk products.

Justice of the Peace Paul Kowarsky upheld the legislation, but said in this case Mr. Schmidt did not break the law because he was distributing to joint owners of cows and not the public at large.

**Ontario's Health Protection and Promotion Act makes it illegal to "sell, offer for sale, deliver or distribute milk or cream that has not been pasteurized or sterilized." Consumption is legal, however.**

The Ontario government will appeal last month’s court ruling that made it legal to distribute raw milk in the province under certain circumstances...The judge had ruled that Mr. Schmidt’s “cow-share” program constituted a legal work-around of the restrictions against selling raw milk. The appeal argues that Justice of the Peace Paul Kowarsky made several legal errors.

Mr. Schmidt, a 54-year-old Durham region dairy farmer and his supporters says milk is healthier before it is pasteurized. **Canadian health officials deem it unsafe for public consumption.**

Mr. Schmidt had argued that if he were found guilty of violating the law, the statutes themselves should be struck down as a violation of his constitutional right to “life, liberty and security of the person.”

Source - National Post, Jan. 21, 2010
Is this the end?