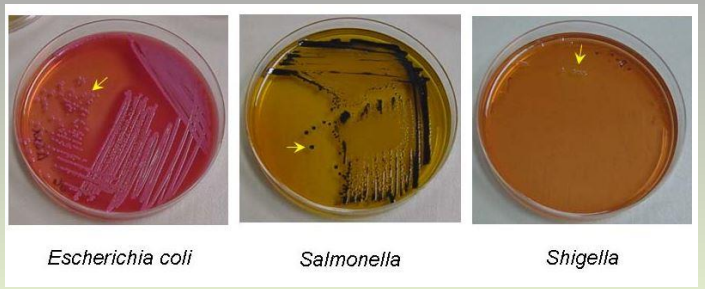
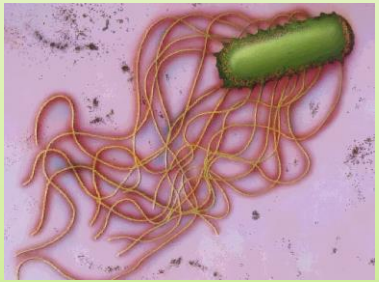


Salmonella - Overview



Prof Charles Ntahonshikira

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ERFAN Laboratory training course, Windhoek October 2022

Presentation layout

- Introduction
- Classification
- Brief history
- The organism
- Sources of Salmonella
- Transmission
- Disease-Salmonellosis
- Diagnostic procedures
- Prevention

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INTRODUCTION

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Genus *Salmonella*

- *Salmonella* spp. are members of the family Entero-bacteriaceae
- They are Gram-negative, facultatively anaerobic rods
- The genus *Salmonella* contains two species, *S. enterica*, the type species, and *S. bongori*.
- *S. enterica* contains six subspecies: ssp. *enterica*, ssp. *salamae*, ssp. *arizonae*, ssp. *diarizonae*, ssp. *houtenae* and ssp. *indica*.

Genus *Salmonella*

- Within each subspecies are serovars; over 2500 serovars are presently known.
- Most of the isolates that cause disease in humans and other mammals belong to *S. enterica* subsp.
- A few serovars, *Salmonella* ser. Typhi, *Salmonella* ser. Paratyphi and *Salmonella* ser. Hirschfeldii are human pathogens that are transmitted from human to human.
- The remaining Salmonella serovars, sometimes referred to as non-typhoidal *Salmonella*, are zoonotic or potentially zoonotic.

Genus *Salmonella*

- Pathogenic salmonella may be:
- Host adapted
  - Human: *S. typhi*
  - Cattle: *S. dublin*
  - Poultry: *S. pullorum*
  - Pigs: *S. choleraesuis*
- Non-host adapted
  - *S. typhimurium*

Genus *Salmonella*; General features

- Salmonella serotypes occur worldwide
  - Infect many mammals, birds, and reptiles
  - Mainly excreted in faeces
  - Ingestion is the main route of infection
  - *Salmonella Enteritidis* infect poultry – organism found in ovaries
  - Salmonellosis – one of the most important food-borne disease

Genus *Salmonella*; Salient features

- Salmonellae are usually motile
- Do not ferment lactose
- Serotyping is based on the Kaufmann and White schema in which somatic (0) and flagellar (H) antigens are identified
- The genus *Salmonella* contains more than 2,500 serotypes
- Occasionally, capsular (Vi) antigens may be detected.
- *Salmonella Enteritidis* infect poultry – organism found in ovaries
- Organisms can be isolated from eggs

CLASSIFICATION

Salmonella; Classification

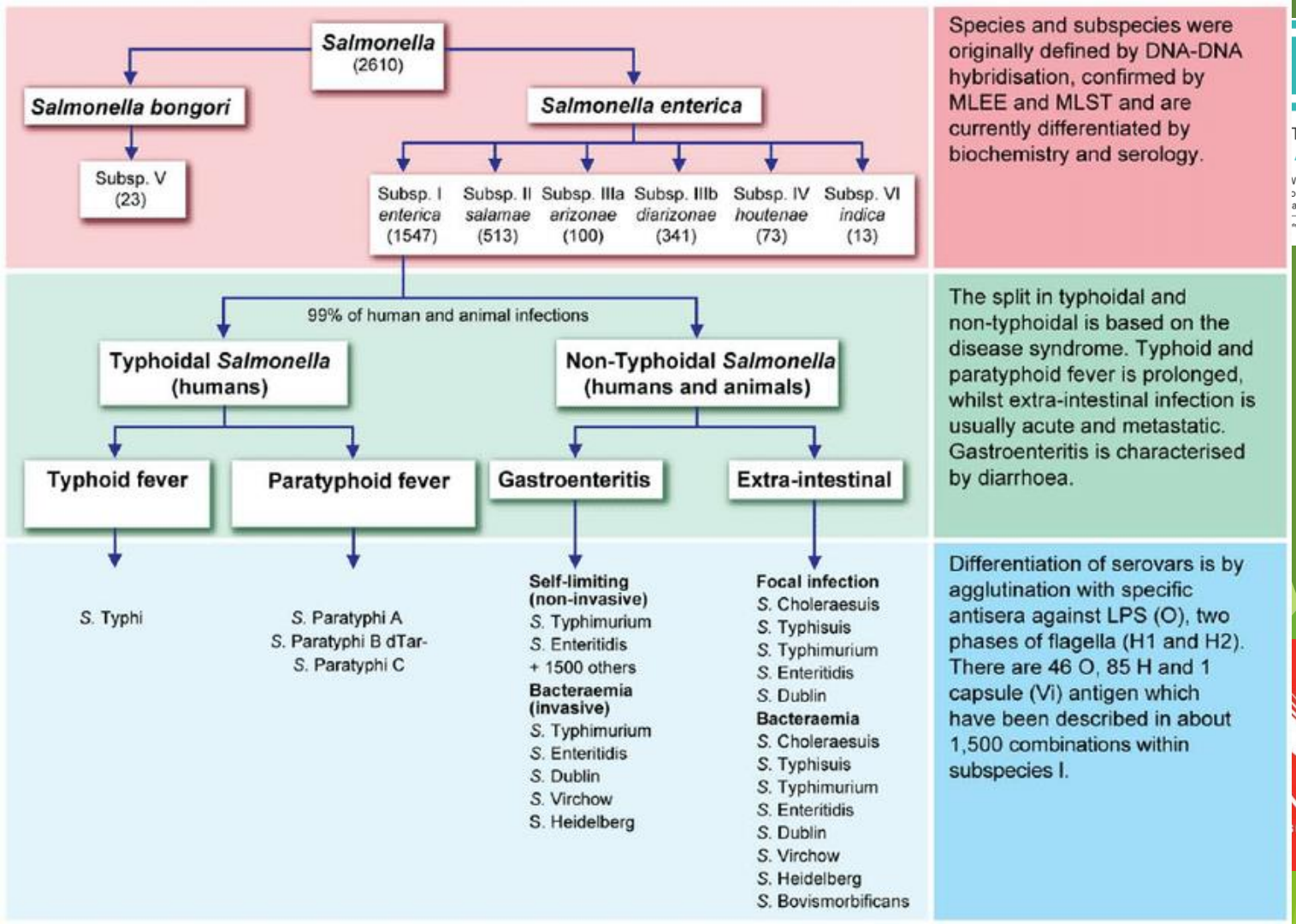
- The taxonomy of the salmonellae has been in flux for many years, and it is problematic, with more than 2500 serotypes.
- Earlier classification system included:
  - **The Kaufmanns-White system**, which identified each serotype as an individual *Salmonella* species,
  - **The Edwards-Ewing system**, which divided the salmonellae into 3 species (*S. choleraesuis*, *S. enteritidis*, *and S. typhi) and hundred of serotypes*
  - **A DNA hybridization scheme** that lumped the salmonellae into two species known as *S. enterica* and *S. bongori*.

Salmonella; Classification

- Under the current American CDC (Center for Disease Control) classification scheme there are two species:
  - *Salmonella enterica*
  - *Salmonella bongori*
- *S. enterica* is further divided into 6 subspecies:
  - *arizonae*
  - *diarizonae*
  - *enterica*
  - *houtanae*
  - *indica* and
  - *salamae*

Salmonella; Classification

- *S. enterica* contains more than 2500 serotypes (2541 in 2004) differentiated on the O and H- Antigens
  - *Salmonella serotype* (serovar) *typhimurium*,
  - *Salmonella* serotype *enteritidis*,
  - *Salmonella* serotype *typhi*,
  - *Salmonella* serotype *paratyphi*,
  - *Salmonella* serotype *cholerae suis* etc.
- Ex.: *Salmonella enterica* subspecies *enterica* serovar Typhi or *Salmonella typhi*



Species and subspecies were originally defined by DNA-DNA hybridisation, confirmed by MLEE and MLST and are currently differentiated by biochemistry and serology.

The split in typhoidal and non-typhoidal is based on the disease syndrome. Typhoid and paratyphoid fever is prolonged, whilst extra-intestinal infection is usually acute and metastatic. Gastroenteritis is characterised by diarrhoea.

Differentiation of serovars is by agglutination with specific antisera against LPS (O), two phases of flagella (H1 and H2). There are 46 O, 85 H and 1 capsule (Vi) antigen which have been described in about 1,500 combinations within subspecies I.

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BRIEF HISTORY

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Salmonella - Brief history

- *Salmonella* was first discovered in 1884 by DANIEL ELMER SALMON
- *D.V.M. (1850-1914); he isolated the bacterium (S. choleraesuis) from the intestine of a pig.*
- By 1980, more than 30,000 people were reported to be infected with *Salmonella* in US.
- This number increased to 42,028 by 1986
- From 1998-2002, the CDC reported An estimated 1.4 million cases occur annually in the U.S.(underreported)

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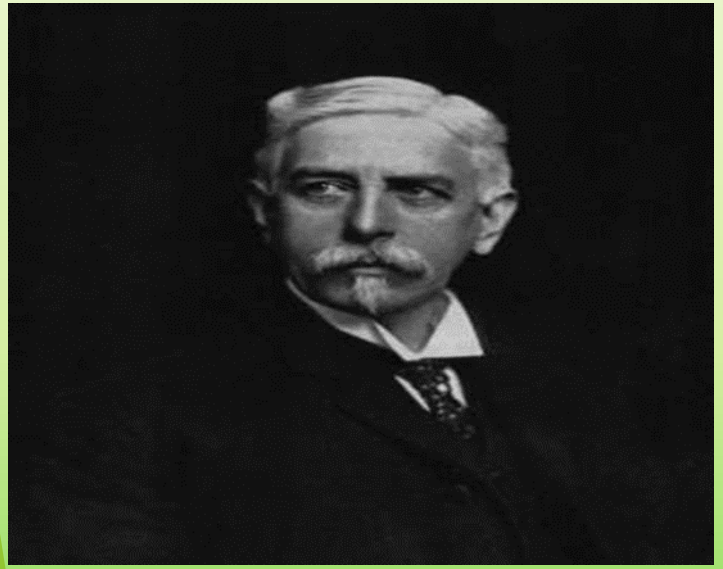
DANIEL ELMER SALMON



- One of the first veterinary students from Cornell University, and holder of the first D.V. M. degree (1876)
- Father of disease eradication
- Pioneer in public health practice and medical research
- Discoverer of salmonellae
- Experimental Immunologist, Epidemiologist
- Administrator - Bureau of Animal Health

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THE ORGANISM

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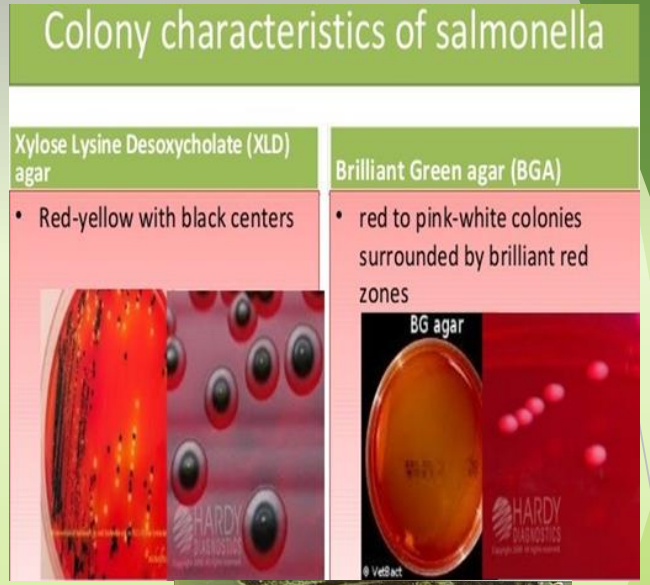
Salmonella - Morphology

- *Gram – negative rods*
- Non-capsulated (except *S. typhi*)
- Non-sporulated
- Peritrichous flagella
- (ensure motility) except *Salmonella gallinarum - Pullorum*

Cultural properties

- Aerobe - facultative anaerobe
- Grow easily on simple culture media
- Onto selective and differential media that contain biliary salts and lactose - grow like lactose-negative “S” colonies.
- Produce H_2S, colonies have a “cat-eye” appearance.
- Selective media: Xylose Lysine Deoxycholate agar (XLD agar), Salmonella Shigella agar (SS agar), Hektoen enteric (HE) medium, brilliant green agar, etc.

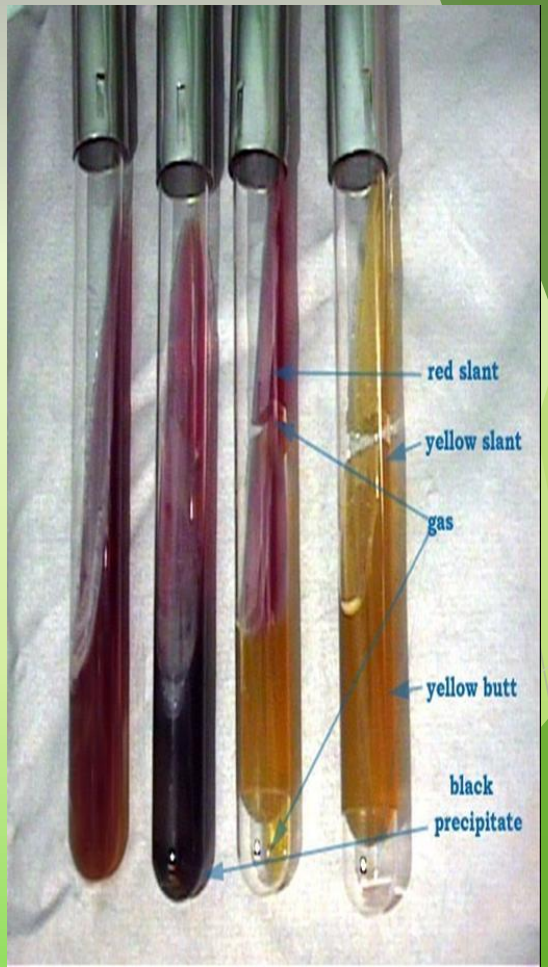


Biochemical Properties

- Indole test negative
- Methyl red test positive
- Voges-Proskauer test negative
- Citrate positive (growth on Simmon's citrate agar)
- IMViC: - + - +
- Motile
- Lactose negative
- Acid and gas from glucose, mannitol, maltose, and sorbitol;
- No Acid from adonitol

Biochemical Properties

- Sucrose, salicin, lactose
  ONPG test negative (lactose negative)
- Lysine decarboxylase positive
- Ornithine decarboxylase positive
- H_2S produced from thiosulfate
- Urease negative
- Gelatin hydrolysis negative
- Phenylalanine and tryptophan deaminase negative



SOURCES OF SALMONELLA

SALMONELLA SOURCES

- Salmonella organisms may be present in:
  - water,
  - soil,
  - animal feeds,
  - raw meat
  - Offal
  - Vegetable
  - RTE products
  - Faecal material is the source of environmental contamination

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TRANSMISSION

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Transmission in humans

- People are often infected when they eat contaminated foods of animal origin
- They can also be infected by ingesting organisms in contaminated food or water.
- Directly transmitted human infections are most often acquired from the feces of reptiles, chicks and ducklings.
- Livestock, dogs, cats, adult poultry and cage birds can also be involved.

Transmission in animals

- *Salmonella* spp. are mainly transmitted by the fecal-oral route.
- They are carried asymptomatically in the intestines or gall bladder of many animals and are continuously or intermittently shed in the feces.
- Vertical transmission occurs in birds, with contamination of the vitelline membrane, albumen, and the yolk of eggs.
- *Salmonella* spp. can also be transmitted in utero in mammals.

Transmission in animals

- Animals may also become infected from contaminated feed (including pastures), drinking water, or close contact with infected animals (including humans).
- Birds and rodents can spread salmonella to livestock.
- Carnivores may also be infected through meat, eggs, and other animal products that are not thoroughly cooked.

The disease – Salmonellosis

Human Salmonellosis

- Intestinal infection with salmonellae can follow one of two infection cycles: One cycle causes **enteritis**, and the other one causes **typhoid**

(a) Enteritis

- Most serotypes cause enteritis, an infection that is limited to the terminal ileum.
- **The salmonellae invade the intestinal wall and produce enterotoxins that cause nausea, vomiting, and diarrhea.**
- Bacteria rarely spread beyond the gastrointestinal wall.
- 8-48 hours after the ingestion of food or drink contaminated with Salmonella, enterocolitis begins with nausea, vomiting abdominal pain, diarrhea which can vary from mild to severe
- In some cases manifestations include fever, headache and chills.

Human Salmonellosis

(b) Enteric fever (Typhoid):

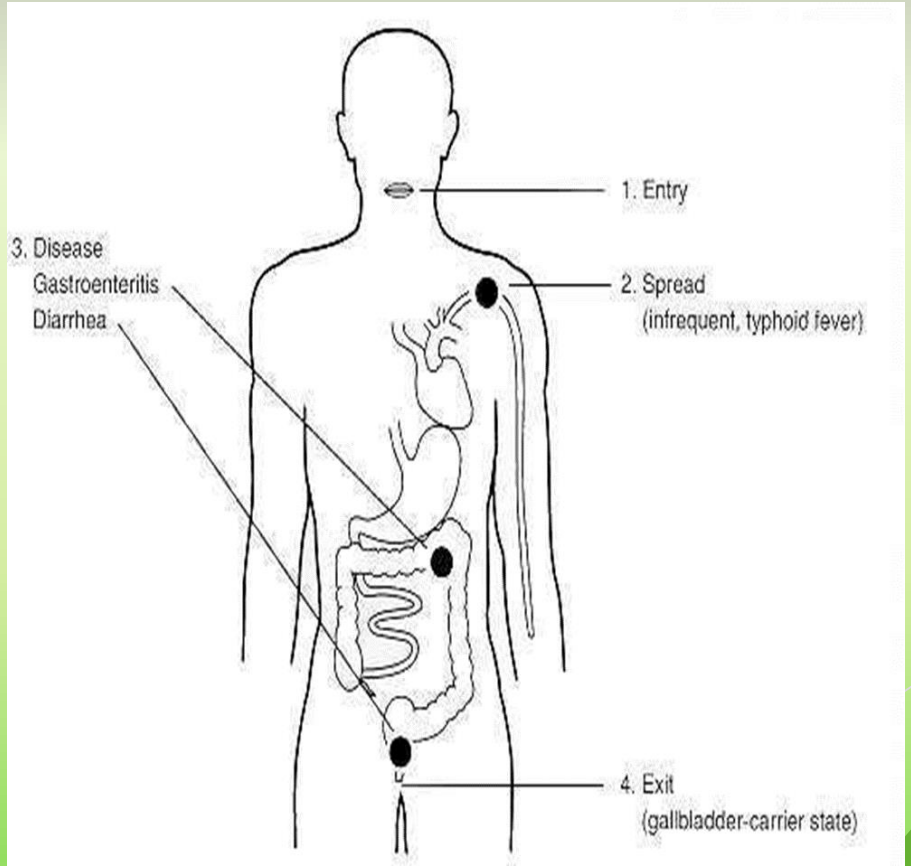
- Two serotypes **Typhi** and **Paratyphi** can cause typhoid.
- The salmonella invade the wall of the terminal ileum and then spread to the intestinal lymphatics, where they are phagocytosed by PMNs and macrophages.
- Salmonella phagocytosed by PMNs are killed, but those phagocytosed by macrophages survive and multiply within phagocytic vacuoles.
- Wandering macrophages that contain salmonellae act as "taxi/cabs" that deliver salmonellae to various reticuloendothelial tissues.
- Infected macrophages are eventually destroyed and salmonellae released from lysed macrophages.

Human Salmonellosis

(c) Primary septicemia

- Patients with anemia may develop septicemia after asymptomatic ileal infection with *S. choleraesuis*
- Manifestation include spiking fever, weight loss, anorexia, anemia, bacteremia, hepatosplenomegaly

The Salmonella infection cycle



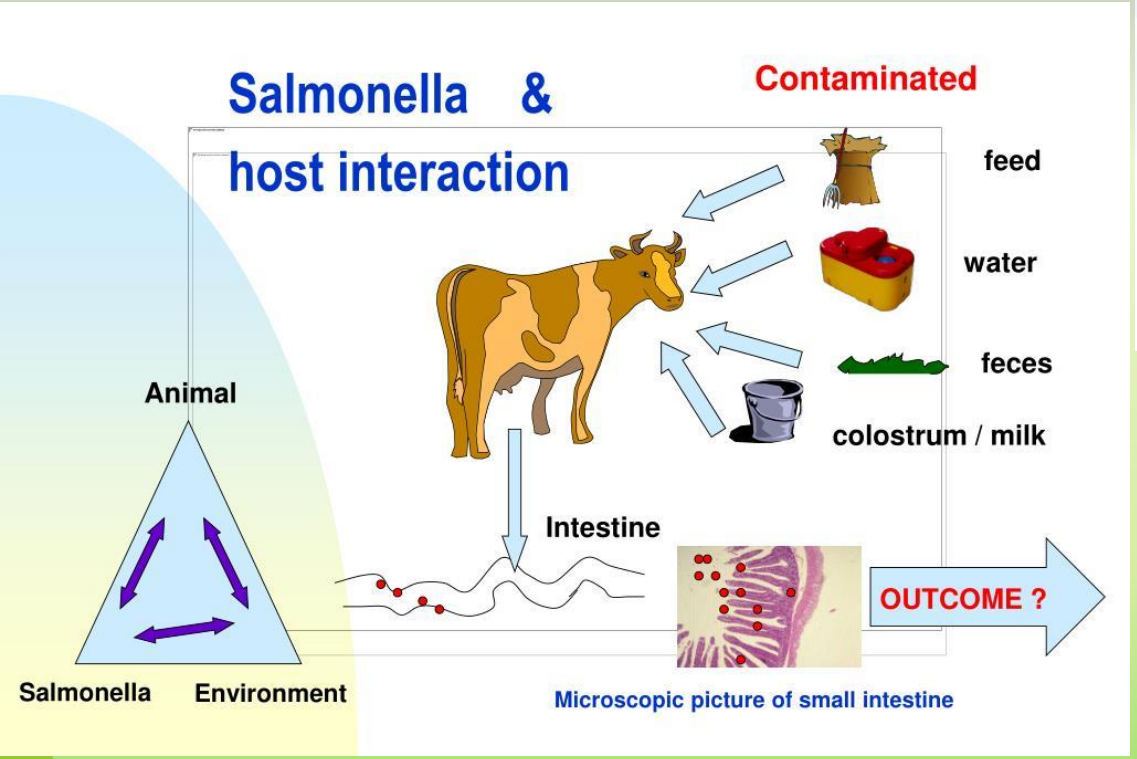
Animal Salmonellosis

- In animals, asymptomatic salmonella infections are common.
- Overall, approximately 1-3% of domestic animals are thought to carry *Salmonella* spp. but the prevalence can be much higher in some species
- Amongmammals, clinical disease is most common in very **young**, **pregnant** or **lactating animals**, and usually occurs after a **stressful event**.
- Outbreaks with a high morbidity rate and sometimes a high mortality rate are typical in **young** ruminants, pigs, and poultry.
- In outbreaks of septicemia, morbidity and mortality can reach 100%.

Animal Salmonellosis

- Estimates of the carrier rate among reptiles vary from 36% to more than 80-90%, and several serovars can be found in a single animal.
- High prevalence rates can also be present in some birds and mammals.
- Young and debilitated or aged animals are particularly susceptible and may develop the septicemic form of the disease.
- In most animal species, both enteric and septicemic forms of salmonellosis are recorded.

Animal Salmonellosis



Salmonella serotypes of clinical importance

Salmonella serotype	Hosts	Consequences of infection
Salmonella Typhimurium	Many animal species Humans	Enterocolitis and septicaemia Food poisoning
Salmonella Dublin	Cattle Sheep, horses, dogs	Many disease conditions Enterocolitis and septicaemia
Salmonella Choleraesuis	Pigs	Enterocolitis and septicaemia
Salmonella Pullorum	Chicks	Pullorum disease (bacillary white diarrhoea)
Salmonella Gallinarum	Adult birds	Fowl typhoid
Salmonella	Turkeys	Arizona or paracolon infection

DIAGNOSTIC PROCEDURES

Isolation and identification

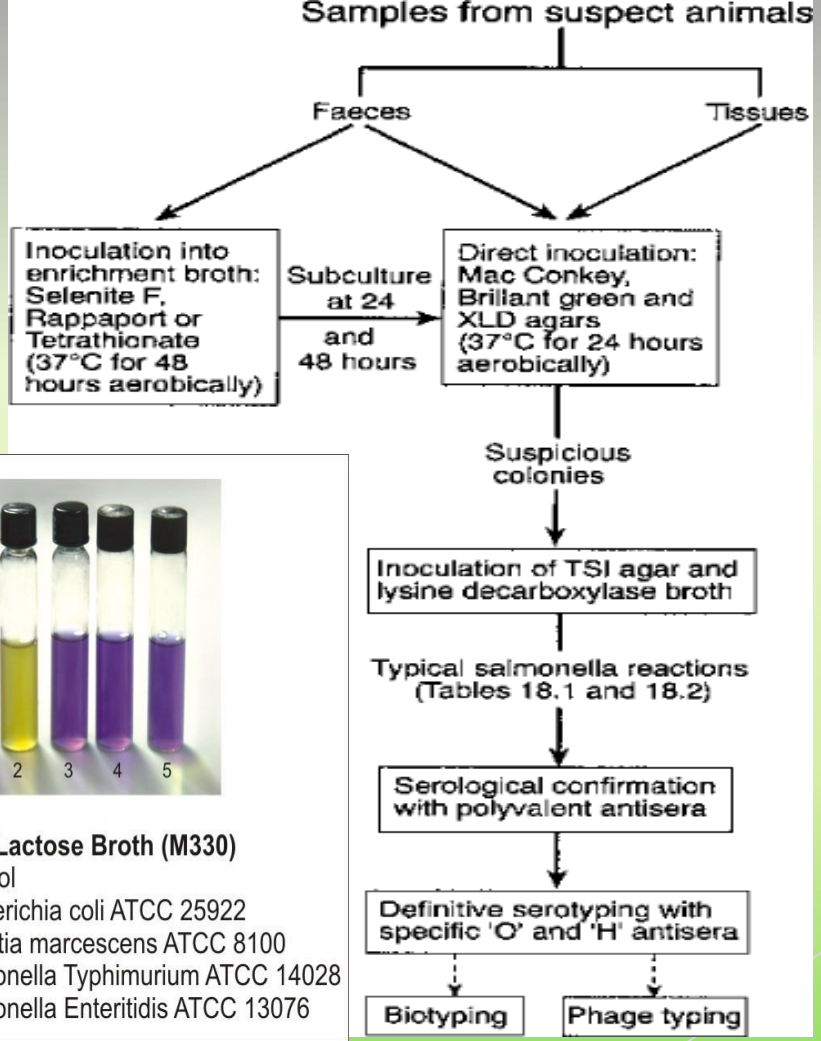
- Non-selective pre-enrichment - Buffered Peptone Water for (BPW)
- Selective enrichment broth/Rappaport-Vassiliadis Medium
- Plating out and identification - Brilliant Green Agar (BGA) as &s Xylose Lysine Desoxycholate Agar (XLD)
  - On BGA medium to change from pink to red.
  - On XLD medium Salmonella varies from black to pink colour with or without black centre.
  - A typical lactose-positive and/or sucrose-positive Salmonella strains produce yellow colonies with or without black centers.

Diagnosis

- A history of previous outbreaks of the disease on the premises, the age group affected and the clinical picture may suggest salmonellosis.
- At postmortem, enterocolitis with blood-stained luminal contents and enlarged mesenteric lymph nodes are commonly observed.
- Laboratory confirmation is required.
- Specimens for submission should include faeces and blood from live animals.
- Intestinal contents and samples from tissue lesions should be submitted from dead animals and abomasal contents from aborted foetuses.

Diagnosis

- Isolation of salmonellae from blood or parenchymatous organs is deemed to be confirmatory for septicaemic salmonellosis.
- A heavy growth of salmonellae on plates directly inoculated with faeces, intestinal contents or foetal abomasal contents strongly suggests the aetiological involvement of the pathogen.
- Recovery of small numbers of salmonellae from faeces is usually indicative of a carrier state.



Lysine Lactose Broth (M330)

1. Control
2. Escherichia coli ATCC 25922
3. Serratia marcescens ATCC 8100
4. Salmonella Typhimurium ATCC 14028
5. Salmonella Enteritidis ATCC 13076

Salmonella Prevention in animals

- Herds and flocks
  - Buy from Salmonella-free sources
  - Isolate new animals
  - All in/all out
- Outbreak
  - Identify carriers
    - ✓Isolate, treat, or cull
  - Retest treated animals
  - Clean and disinfect

Salmonella
Prevention in animals

- Preventing clinical disease
  - Good hygiene
  - Minimize stressful events
  - Colostrum
  - Vaccination
    - ✓Also reduces colonization and shedding
  - All reptiles are a source

Salmonella
Prevention in humans

- Food-borne diseases
  - Avoid raw or undercooked eggs, poultry, meat; unpasteurized milk/dairy
  - Wash foods before eating
  - Avoid cross-contamination of food
    - ✓Keep uncooked and cooked foods separately
    - ✓Wash hands and kitchen tools
  - Do not feed infants or change diapers while handling food

Salmonella Prevention in humans

- Animal contact
  - Wash hands after contact
  - If immunocompromised, avoid contact with reptiles, young chicks, ducklings
- Reptiles
  - Children under 10 years of age
  - Wash hands, cages, and surfaces
  - Change clothes
  - Supervision
  - Do not allow reptiles to roam freely

THANK YOU!

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