



2nd Professional Year (Veterinary Microbiology)

Topic: Salmonella

Dr Manoj Kumar

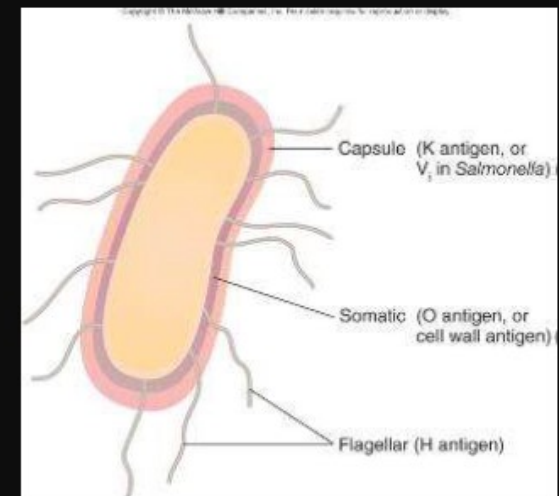
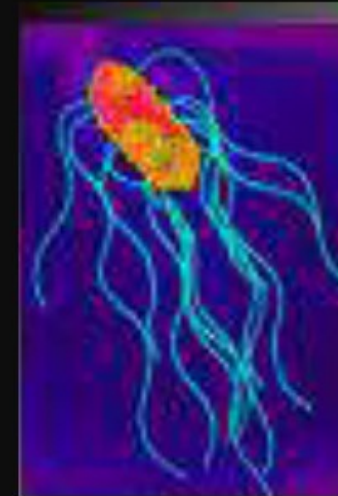
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History

- The genus *Salmonella* was named after Daniel Elmer Salmon, an American veterinary pathologist.
- Theobald Smith was the actual discoverer of the bacteria that causes hog cholera (*Salmonella enterica* var. *Choleraesuis*)
- First isolated in 1884
 - *S. choleraesuis* in pig intestine



Classification and Taxonomy of *Salmonella* (Confused)

- Old: Serotyping & biochemical assays used to name individual species within genus (e.g., *Salmonella enteritidis*, *S. choleraesuis*, *S. typhi*)
 - Over 2600 O-serotypes (referred to as species) (Kauffman-White antigenic schema)
 - Bioserotyping (e.g., *S. typhimurium*)
- New: DNA homology shows only two species *Salmonella enterica* (six subspecies) and *S. bongori*
 - Most pathogens in *S. enterica ssp. enterica*

A microscopic image showing several Salmonella bacteria. The bacteria are rod-shaped, pinkish-red, and have long, thin, wavy flagella extending from their ends. They are scattered across a light blue background.

Species of Salmonella

- Two species
 - *S. bongori*
 - *S. enterica*
 - Six subspecies

SALMONELLA - CLASSIFICATION

- *Salmonella enterica* spp. is subdivided into 6 subspecies:
- *enterica* (I) [99% of *Salmonella* isolated from humans]
- *salamae* (II)
- *arizonae* (IIIa)
- *diarizonae* (IIIb)
- *houtenae* (IV) and
- *indica* (VI)

SALMONELLA - CLASSIFICATION

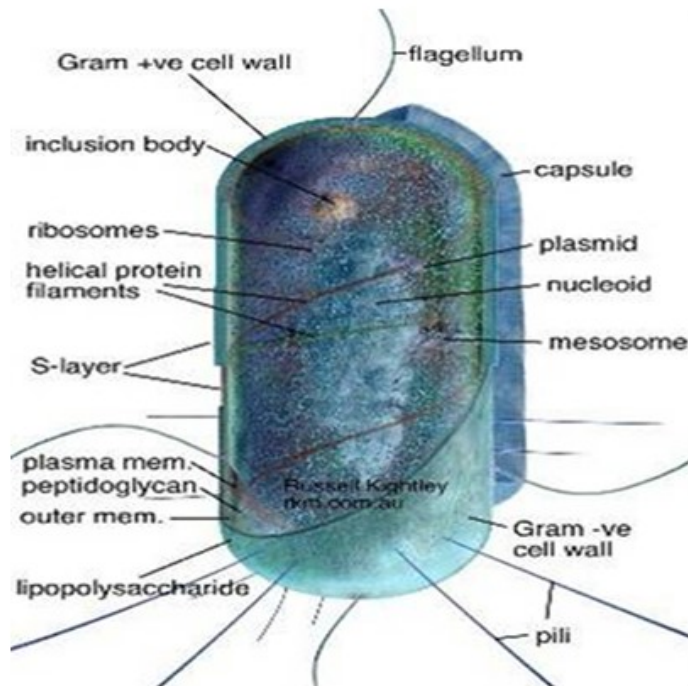
- **SEROVARS / SEROTYPES**

- A **serotype** or **serovar** is a distinct variation within a species of bacteria or virus or among immune cells of different individuals.
- These microorganisms, viruses, or cells are classified together based on their cell surface antigens, allowing the epidemiologic classification of organisms to the sub-species level.
- The *Salmonella* has been determined to have over 2600 serotypes.

SALMONELLA - CLASSIFICATION

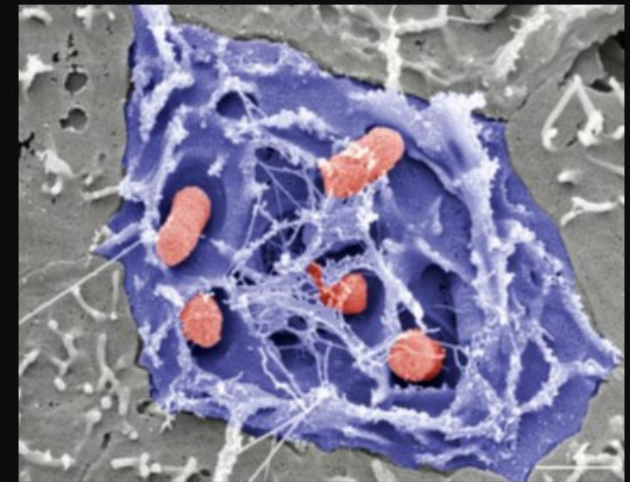
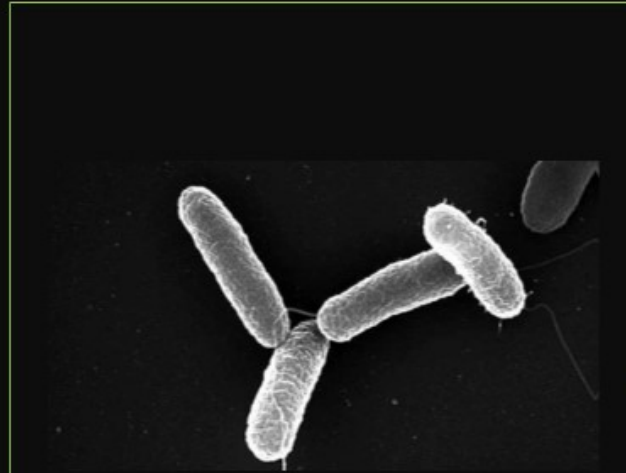
- *S. enterica* subsp *enterica* is further divided into several serotypes or serovars based on biotypes (**Antigenic structures**)
- For e.g.
 - *S. enterica* subsp *enterica* ser. Typhimurium
 - *S. enterica* subsp *enterica* ser. Paratyphi
 - *S. enterica* subsp *enterica* ser. Typhi


Morphology & General Characteristics of Salmonella



- Coliform bacilli (enteric rods)
- Size varies from 2-4 X 0.6 μ m
- Flagellated. (Except *S. gallinarum* & *S. pullorum*)
- Motile gram-negative facultative anaerobes
- Resistant to bile salts
- H₂S producing
- Non Spore Formers.
- Facultative Anaerobic Bacilli.
- Chemoorganotrophic.

Electron Micrograph
of *Salmonella*
typhimurium



- 
- Salmonella grow at an optimum of 37°C
 - Optimum pH for growth of Salmonella is 6.5-7.5
 - may grow at a pH range of 4.5-9.0

CULTURAL CHARACTERISTICS

- **NUTRIENT BROTH**

- After overnight incubation at 37 degree C, uniform turbidity will be produced in the medium.

- **NUTRIENT AGAR MEDIUM**

- Greyish white, circular colonies will be formed at 2-3mm diameter.

- **BLOOD AGAR MEDIUM**

- Circular, smooth, convex colonies will be formed

- **MACCONKEY AGAR MEDIUM**

- They produce pale yellow or colorless colonies with 1-3 mm diameter in size.

- **WILSON&BLAIR BISMUTH SULPHITE MEDIUM**

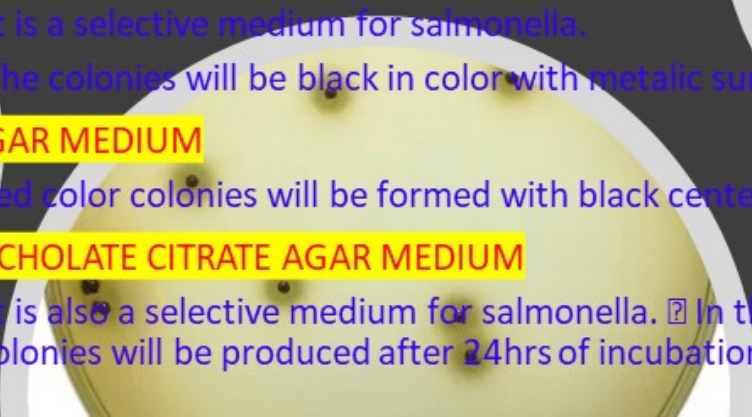
- It is a selective medium for salmonella.
- The colonies will be black in color with metallic surface appearance.

- **XLD AGAR MEDIUM**

- Red color colonies will be formed with black centers.

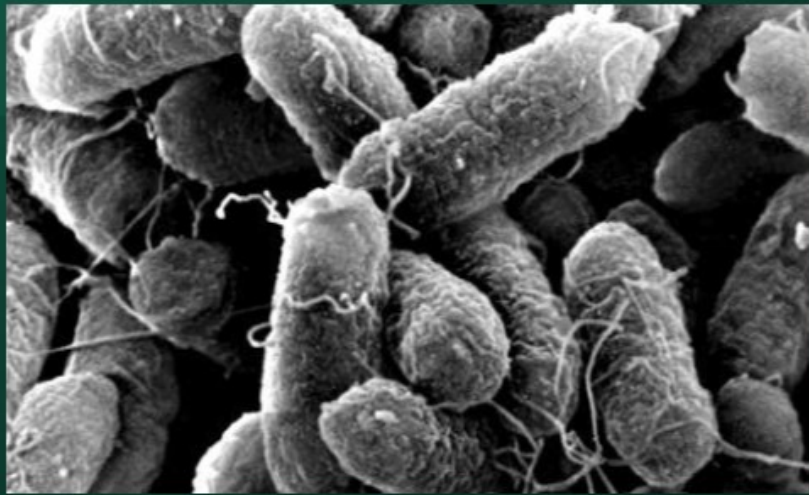
- **DEOXYCHOLATE CITRATE AGAR MEDIUM**

- It is also a selective medium for salmonella. In this colorless colonies will be produced after 24hrs of incubation

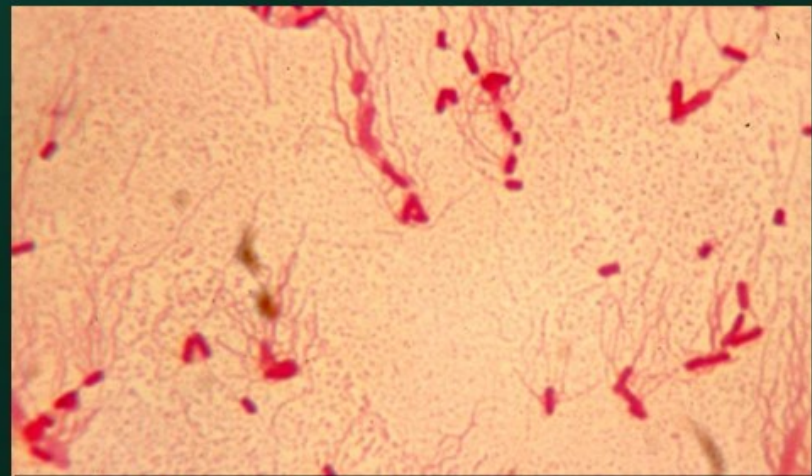


Biochemical properties:

- **H₂S production**
- Lactose negative,
- Urease negative
- Indole negative



S. enterica subsp *enterica* ser. Typhi under Scanning Electron Microscope

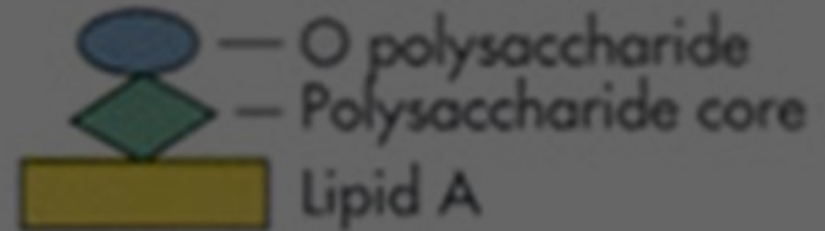


Salmonella with a peritrichous arrangement of flagella

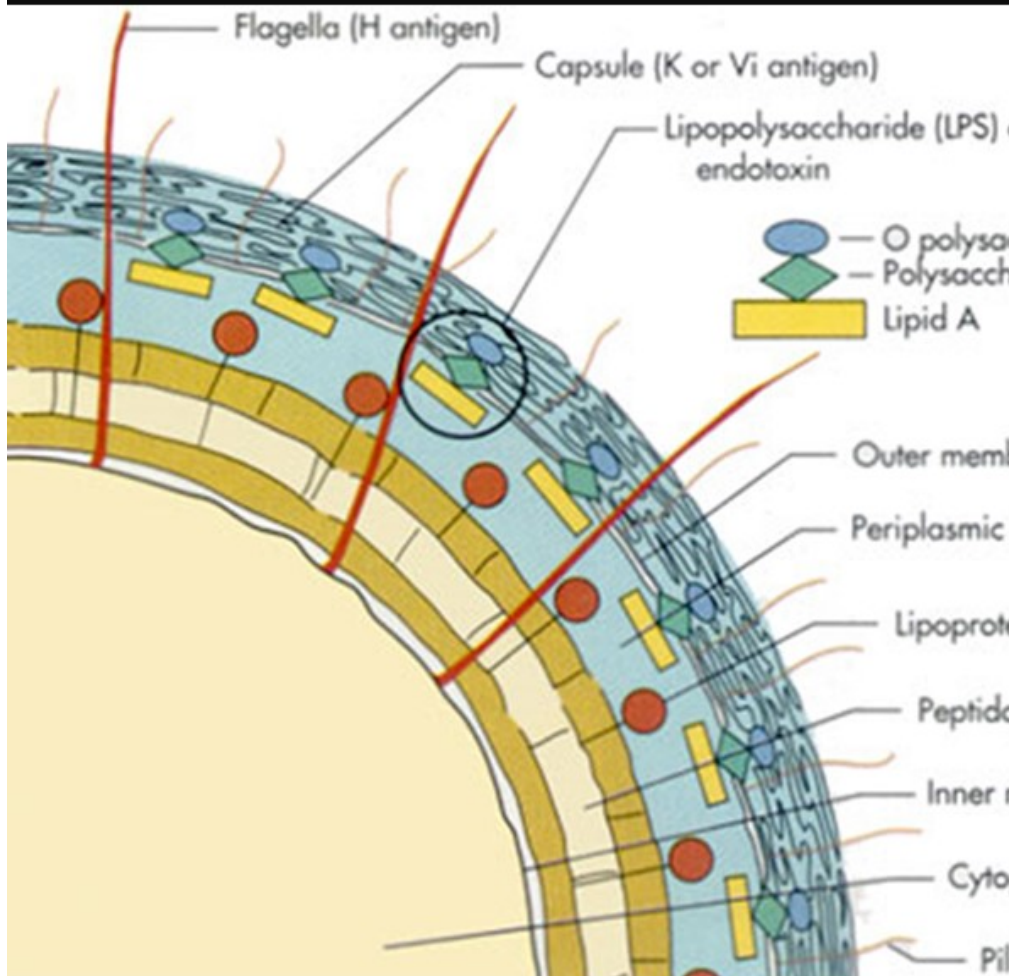
Antigenic structure

- Based on the Kauffman-White scheme of classification: serogroups or serotypes based on O and H (flagella) antigens.
- The O antigens
 - O antigens are vary variable
 - Mosaic structure
 - Epitope combination, special antigens of the isolates
- The H (flagella) antigens
 - H antigens has a mosaic structure as well
 - occur in two phases;
- 1 and 2 and only one phase is expressed at a given time
- Salmonella typhi also has a Vi antigen which is a capsular antigen.

ENDOXYN



ANTIGENIC STRUCTURE



The genus *Salmonella* has three kinds of major antigens with diagnostic or identifying applications:

Somatic, surface, and flagellar.

Somatic (O) or Cell Wall Antigens.

Flagellar (H) Antigens.

Surface (Vi) Antigens. (Found in some spp.)

ANTIGENIC STRUCTURE

- **Somatic (O) or Cell Wall Antigens.**
- Occurs on the surface of the outer membrane and are determined by the specific sugar sequences on the cell surfaces.
 - Integral part of the cell wall [**LPS complex**].
 - Heat stable- resistant to boiling up to **2hrs ; 30 mins.**
 - Resistant to **96%** alcohol at **37oC; 4 hrs.**
 - Generally O- antigen is less antigenic than H-antigen.
 - Mosaic of two or more antigenic factors.

ANTIGENIC STRUCTURE

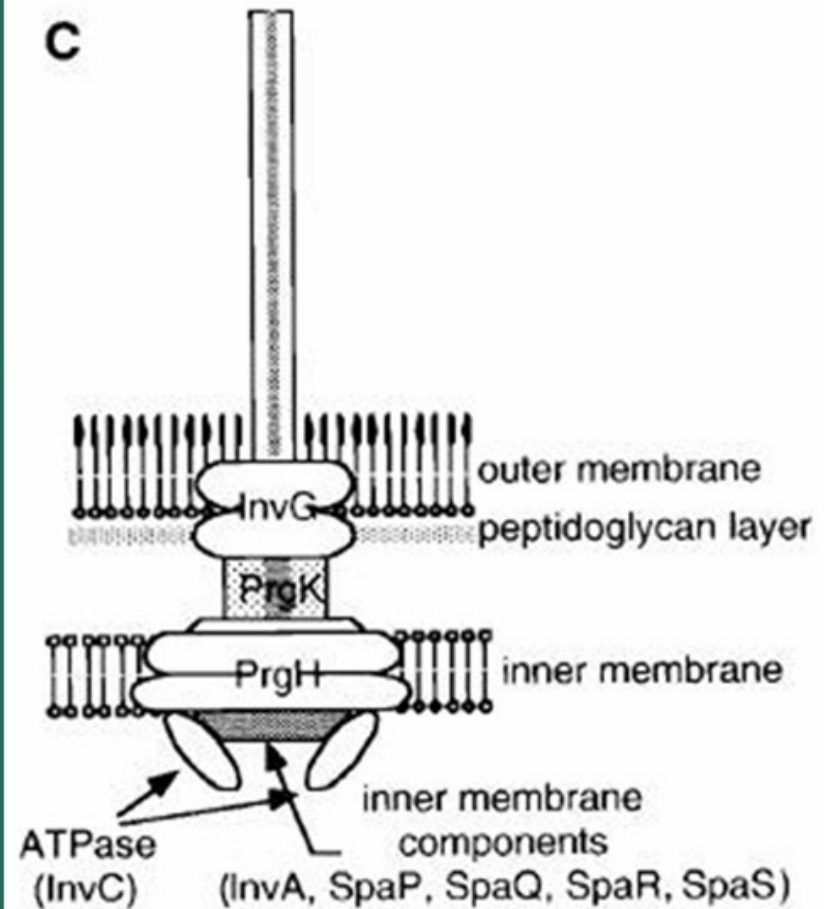
- **Flagellar (H) Antigens**
 - Present on flagella, Heat and alcohol labile.
 - H- antigen of salmonella are genus specific and are not share with other enterobacteria.
- Strongly immunogenic and associated with the formation of antibodies following infections and immunization.

ANTIGENIC STRUCTURE

- **Surface (Vi) Antigens**
 - Many strains of *S. typhi* fail to agglutinate with O antiserum when isolated freshly; due to the presence of
- **Surface polysaccharide antigen** enveloping O antigen.
 - Heat labile, tends to be lost on serial sub- culturing.
 - Act as a virulence factor inhibiting phagocytosis & resisting complement activation and bacterial lysis by the alternate pathway and peroxidase mediate killing.

VIRULENCE FACTORS

- Endotoxin – may play a role in intracellular survival
- Capsule (for *S. typhi* and some strains of *S. paratyphi*)
- Adhesions – both fimbrial and non-fimbrial
- Type III secretion systems and effector molecules
 - Involved in promoting entry into intestinal epithelial cells
 - Involved in the ability of *Salmonella* to survive inside macrophages
- Outer membrane proteins - involved in the ability of *Salmonella* to survive inside macrophages
- Flagella – help bacteria to move through intestinal mucous
- Enterotoxin - may be involved in gastroenteritis
- Iron capturing ability



Methods That Circumvent Phagocytic Killing

METHOD	EXAMPLE
Inhibition of phagolysosome infusion	<i>Legionella</i> species, <i>Mycobacterium tuberculosis</i> , <i>Chlamydia</i> species
Resistance to lysosomal enzymes	← <i>Salmonella typhimurium</i> , <i>Coxiella</i> species, <i>Ehrlichia</i> species, <i>Mycobacterium leprae</i> , <i>Leishmania</i> species, <i>Salmonella typhi</i>
Adaptation to cytoplasmic replication	<i>Listeria</i> species, <i>Francisella</i> species, <i>Rickettsia</i> species

HOST SPECIFICITY

- Wide host specificity
 - normal flora in the GI of animal
 - SALMONELLOSIS-GASTROENTERITIS in human; zoonosis
 - *S. enteritidis*, *S. typhimurium*
- Adapted to animal
 - Serious infection in animal
 - focal infection in human (mainly kids)
 - *S. choleraesuis*
- Adapted to human
 - only in human: ENTERIC FEVER
 - *S. typhi*, *S. paratyphi* A, B, and C

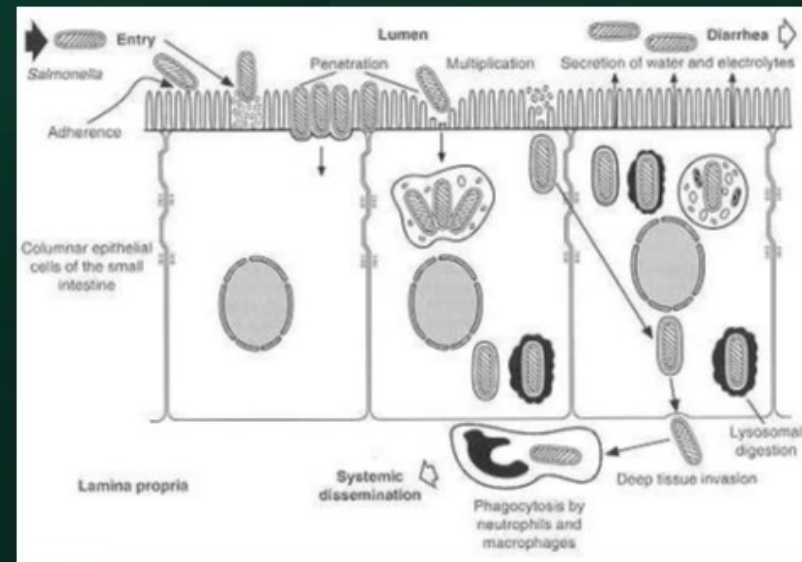
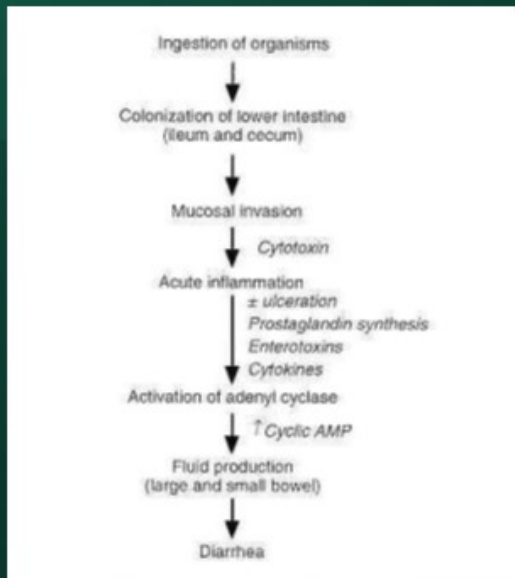
PATHOGENICITY

- Salmonella are strict parasites of Humans as well as animals.
- *S. typhi*, *S. paratyphi A*, *S. paratyphi B* are confined to humans.
- Some species are host adapted
- For e.g.
 - *S. abortus-equi* found only in horses.
 - *S. abortus-ovis* only in sheep.
 - *S. gallinarum* in poultry.
 - *S. typhimurium* have a wide range of hosts affecting animals humans and birds.

Pathogenicity

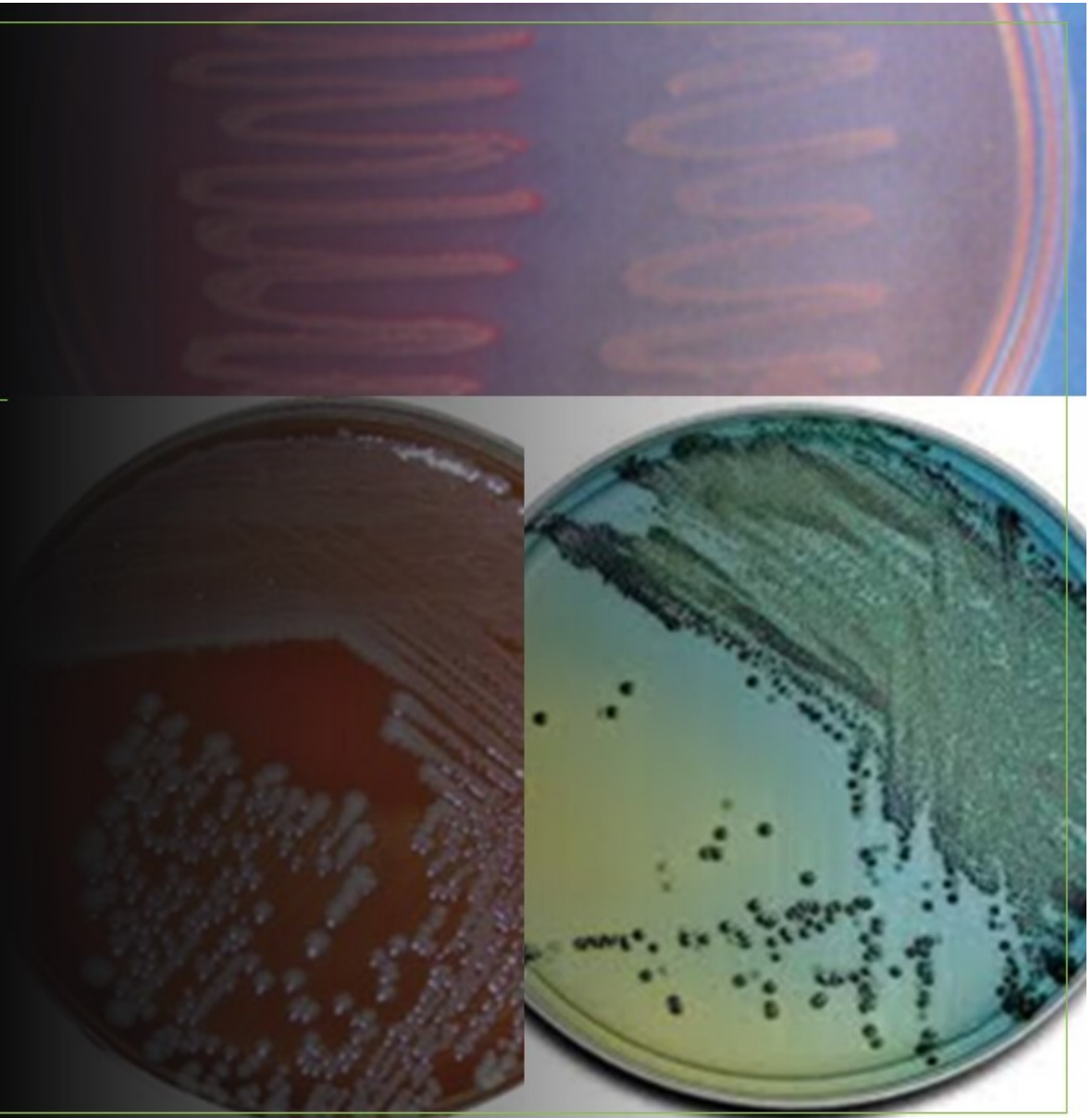
- Disease depends on age of the host
 - more severe in newborns, infants, the elderly
- Disease also depends on the serovar and type of host
 - Host specific serovars: typhi, paratyphi cause disease only in humans
 - pullorum/gallinarum in poultry, dublin in cattle;
 - choleraesuis serovars in pigs can also infect humans;
 - typhimurium and enteritidis are the major serovars that cause
- disease in humans, cattle, poultry, sheep, pigs, horses, and wild rodents.

SALMONELLA Infection - Pathogenesis



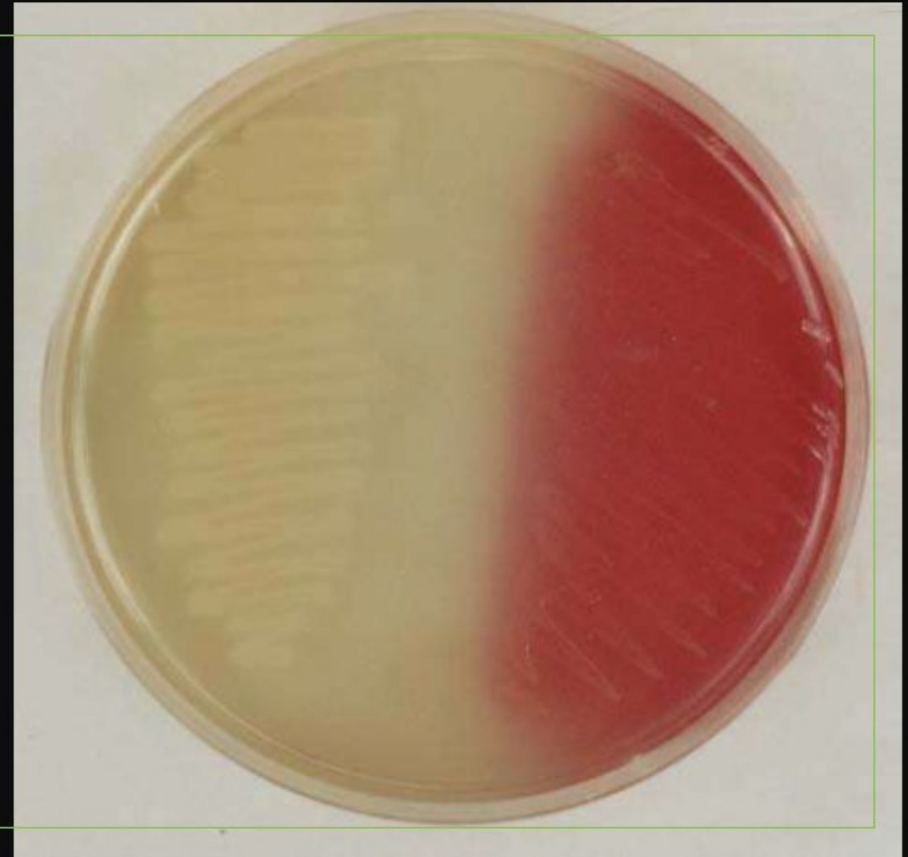
Microbiological diagnosis

- **Microscopic examination:**
 - Direct smear has no value in the diagnosis.
- **Culture:**
 - Samples are inoculated onto
 - Blood agar
 - brilliant green and/or bismuth sulphite selective
 - Hektoen Enteric (HE) agar
 - differentiating media.



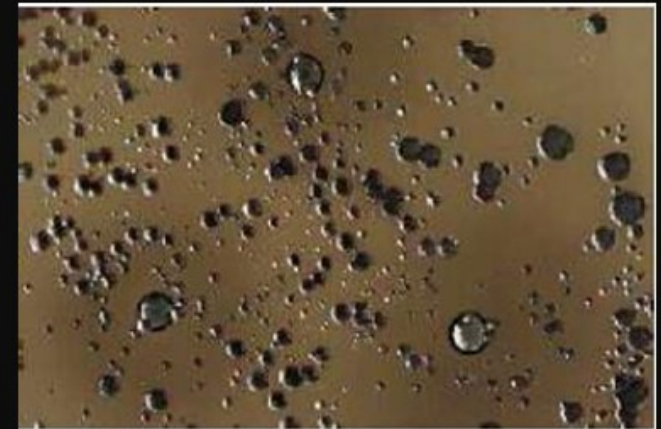
Brilliant-green culture media

- brilliant green – for selection
- lactose, dextrose, saccharose
- Andrade indicator
- (acidic pH → -
- *Salmonella* lactose neg.– no colour
- - *E. coli* lactose+ - red)

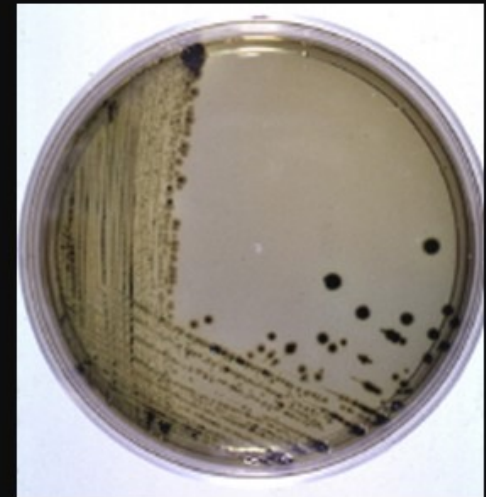


Bismuth-sulphite media

- brilliant green – for selection
- bismuth salt + sodium sulphite
- → *Salmonella* H₂S production
- ⇒ Bismuth sulphide (black)

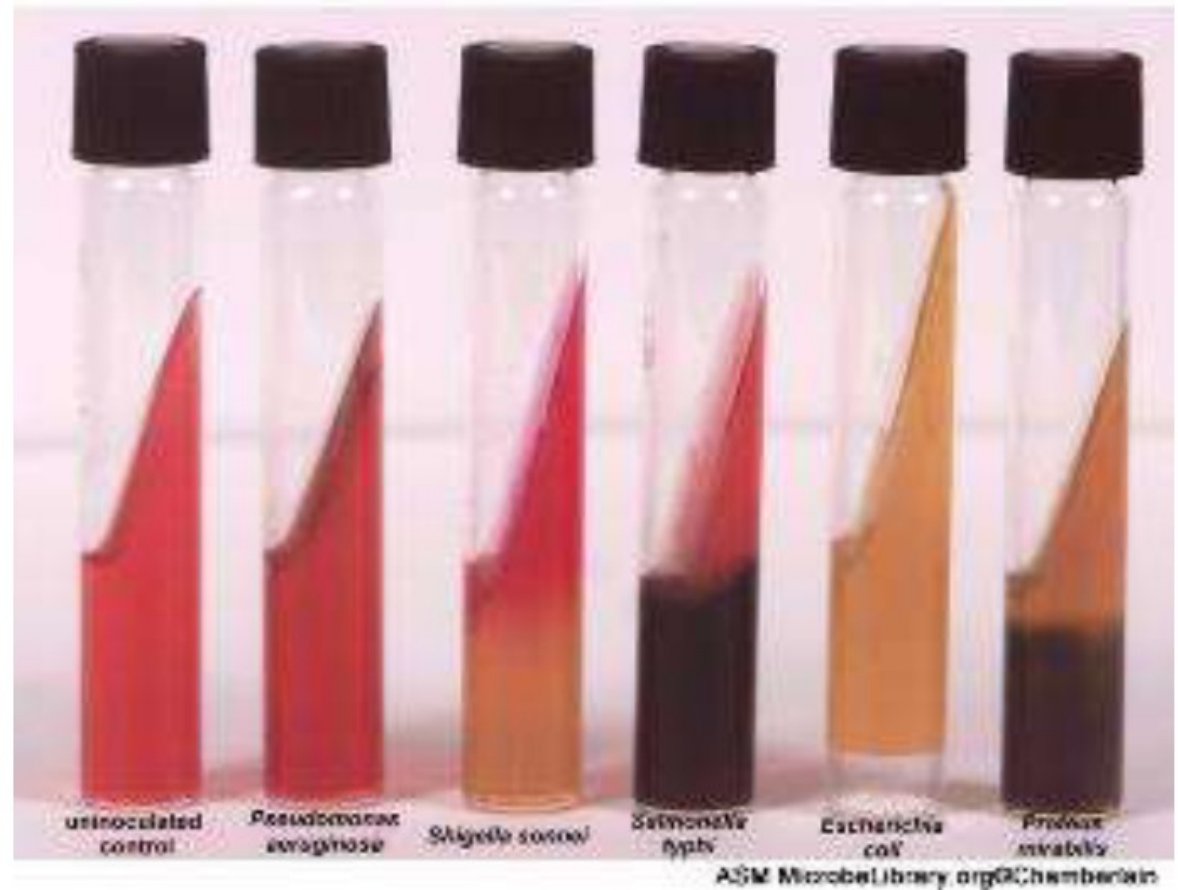


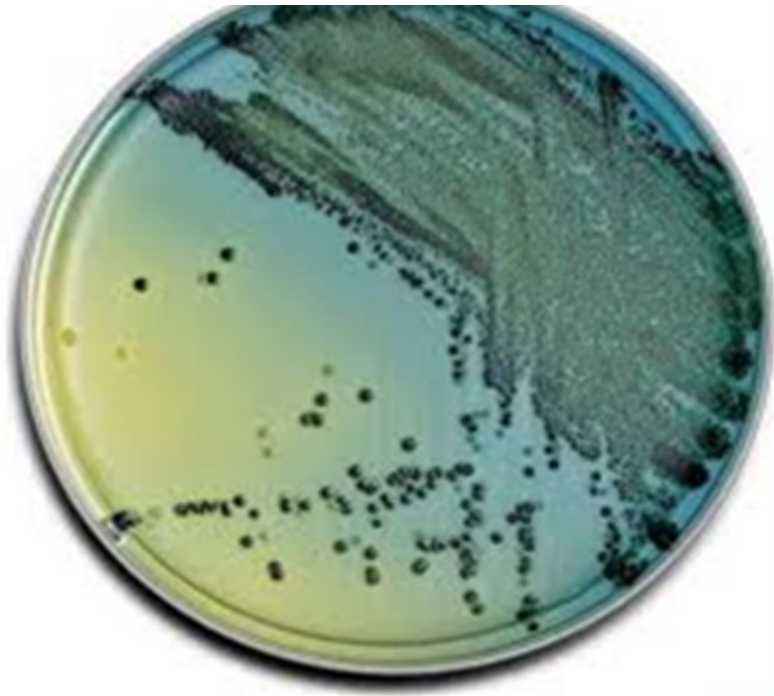
- Black colonies on Hectoen-Enteric agar.
- Wellcolex serogrouping determination for *Salmonella enterica* subspecies enterica serogroup B .
- Antimicrobial susceptibility testing



Biochemical identification:

- Lactose (-)
- dextrose fermentation
- gas formation
- H₂S (+)





- Colony morphology
- Biochemical tests
- Antibody-based serotyping tests

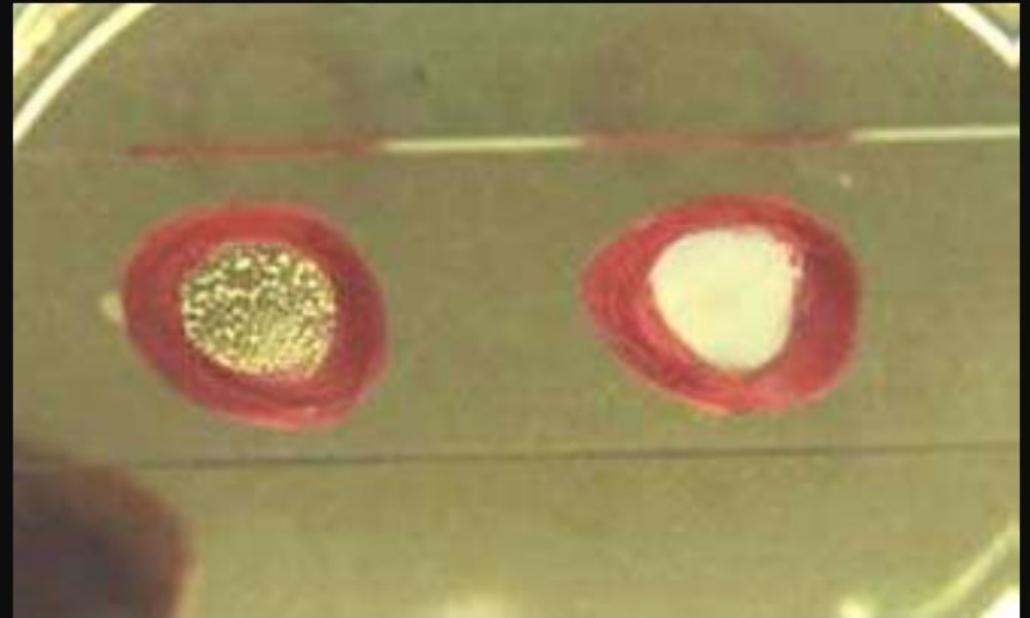


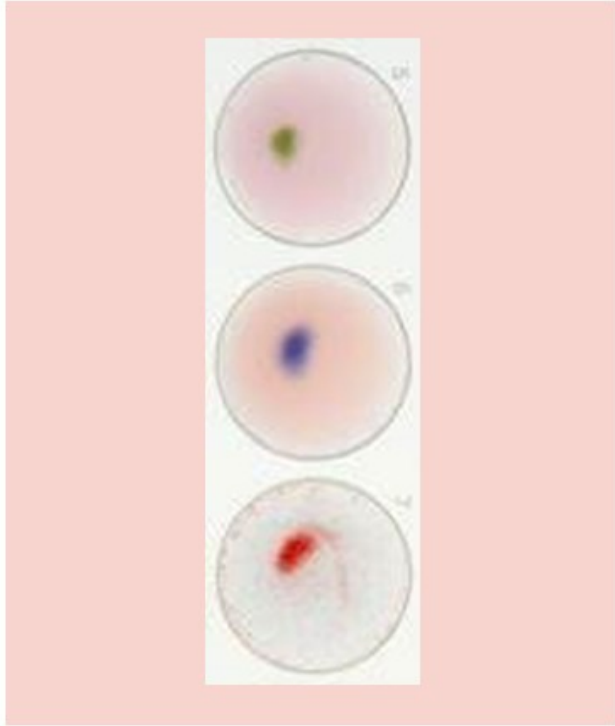
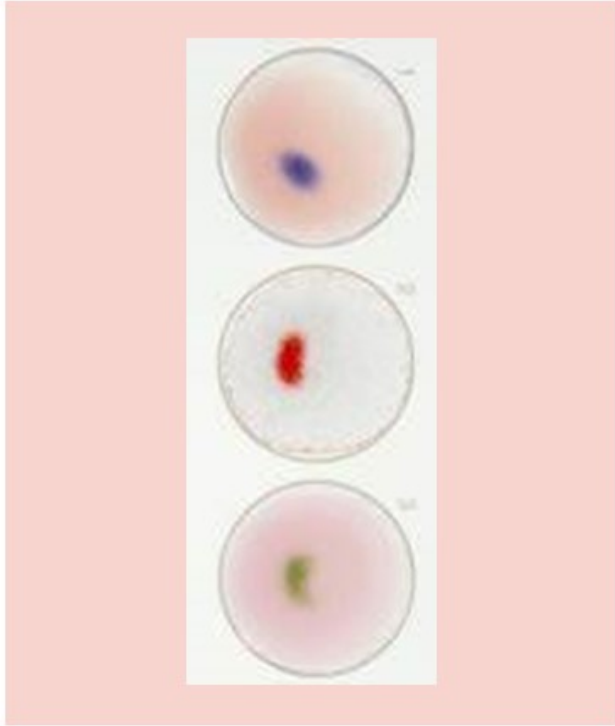


API 20E by bioMerieux

Serological identification:

- Slide agglutination with specific
 - antibodies to detect presence
 - the O and H antigens on the
 - bacteria culture
-






- **Latex reagent 1:**
- Green latex (anti-*Salmonella* grp. D₁ Abs)
- Blue latex (anti-*Salmonella* grp. C Abs)
- Red latex (anti-*Salmonella* grp. B Abs)

- **Latex reagent 2:**
- Blue latex (anti-*Salmonella* grps. E & G Abs)
- Red latex (anti-Vi Abs)
- Green latex (anti-*Salmonella* grp. A Abs)



Bacteriophage typing

- the surface of the plate is inoculated with a broth culture of the investigated strain
 - a number of phages are spotted on the plate
 - after incubation the phage type is determined by the pattern of lyses
-



Molecular methods

– PCR (arbitrary primed (AP)-PCR, ERIC-PCR)

– Pulsed-field gel-electrophoresis (PFGE)

– rRNA sequencing

Pathogenicity in animals

- Prevalent in all species viz.,
 - Mammals
 - Bird
 - Reptiles
 - Amphibians
 - Fish
- Invertebrates
- Some serovars have narrow host range
- Enteritis in Ruminants, Pigs, Horses
- Septicaemia in young animals.



Pathogenicity of Salmonella in animals

- Salmonellae often localize in the mucosae of infected animals viz, .
 - Ileum
 - caecum
 - Colon
 - mesenteric lymph nodes
- Subclinical infection - may persist with shedding of small numbers of salmonellae in the faeces.
- Latent infections- salmonellae remain present in the gall bladder; do not excreted.
- Clinical disease - may develop from subclinical and latent infections when animals are stressed.

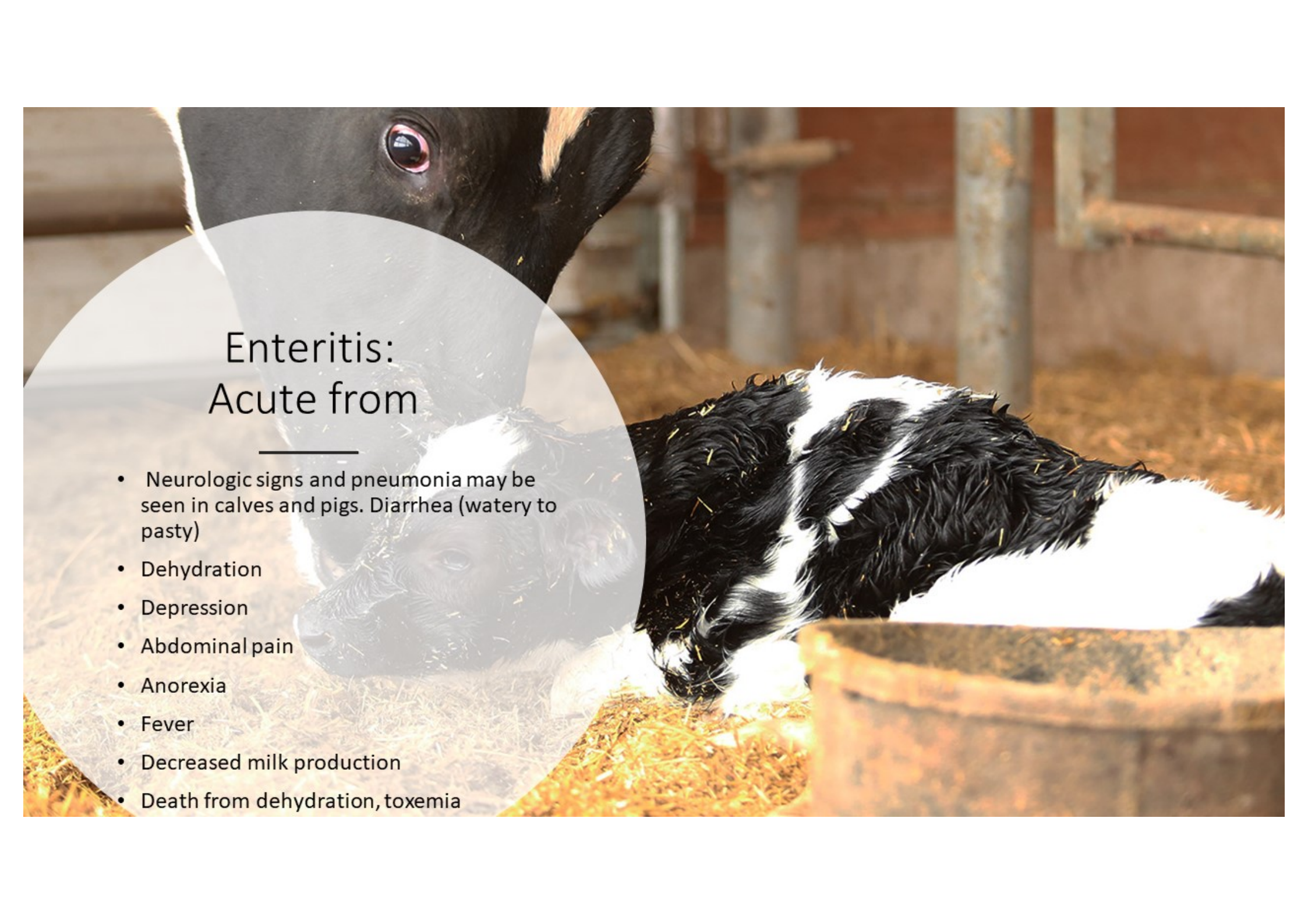
Transmission in animals

- •Fecal-oral
- –Carried asymptotically
- •Fomites, mechanical vectors
- •Vertical
- –Birds
- •*In utero*
- •Contaminated food and water






- Young calves, piglets, lambs, and foals may develop both enteritis and the septicemic form.
- Adult cattle, sheep, horses, dogs, cats, and other mammals may develop acute enteritis when exposed to an sufficiently large dose of a virulent strain.
- Chronic enteritis may develop in growing pigs and occasionally in cattle.

A black and white cow is lying down in a barn, with its head resting on a wooden trough. The cow's eye is visible, and it appears to be resting or possibly unwell. A semi-transparent circular overlay is positioned over the cow's head and neck area, containing text and a list of symptoms. The background shows the interior of a barn with wooden walls and metal bars.


Enteritis: Acute from

- Neurologic signs and pneumonia may be seen in calves and pigs. Diarrhea (watery to pasty)
- Dehydration
- Depression
- Abdominal pain
- Anorexia
- Fever
- Decreased milk production
- Death from dehydration, toxemia

A black cow is standing in a stall. The floor is covered with straw bedding. A metal bucket is on the floor near the cow. The background shows a white wall and some hay. A large, semi-transparent circular graphic is overlaid on the left side of the image, containing text.

Enteritis: Subacute & chronic from


- Subacute
- Occurs in adult
 - Diarrhea
 - Weight loss
- Chronic
- Occurs in adult
 - Adults, older calves, growing pigs
 - Emaciation, fever, inappetence, scant feces



Septicemia


- Young animals
 - Very young calves
 - Lambs, foals
 - Pigs up to 6 months
- Clinical signs
 - Depression, fever
 - CNS signs or pneumonia (calves, pigs)
 - Dark discoloration of skin (pigs)
- Death 1 to 2 days





Abortion

- Associated serovars
 - Dublin (cattle)
 - Abortusovis (sheep)
 - Abortusequi (horses)
 - May be first clinical sign in cows with subacute enteritis
- Joint infections/gangrene

A close-up photograph of a dog's fur, showing a mix of light brown and white hairs. A portion of the dog's ear is visible in the upper center. The background is blurred, suggesting an outdoor setting with greenery.

Clinical Signs: Dogs and Cats

- **Acute diarrhea with septicemia** - seen occasionally in puppies and kittens or in adults stressed by concurrent disease
- Vomition
- Weight loss
- Pneumonia may be evident
- Chronic enteritis - abortion may occur in pregnant dogs, cats and live progeny may have enteritis
- Conjunctivitis in infected cats.



THANK YOU FOR
THE ATTENTION!

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