Family- Enterobacteriaceae

Domain	Bacteria
Phyum	Proteobacteria
Class	Gamma proteobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	Escherichia
Genus	Salmonella
Genus	Shigella
Genus	Citrobacter
Genus	Enterobacter
Genus	Edwardsiella

Genus	Klebsiella
Genus	Morganella
Genus	Proteus
Genus	Providencia
Genus	Serratia
Genus	Yersinia

- Most members of the family *Enterobacteriaceae* share the following characters
- Gram negative, non sporing rods, often motile, usually by means of peritrichate flagellae, capsulate or non capsulate.
- Easily cultivable on ordinary laboratory media. Aerobic and facultative anaerobic.
- All species ferment glucose with the formation of acid or of acid and gas. Reduce nitrate to nitrite with exception of some strains of *Erwinia* and *Yersinia*.
- Oxidase negative, catalase positive except *Shigella dysenteriae*
- They are at present 28 genera and more than 80 well defined species in the *Enterobacteriaceae* (not including the large number of *Salmonella* serotypes/species) have been recognized.
- Note: The term Coliform or Coliform bacteria is used to refer to those members of *Enterobacteriaceae* that usually ferment lactose, such as *E.coli, Klebsiella* and *Enterobacter*species.
- Based on the pathogenicity the *Enterobacteriaceae* can be divided into 3 groups
    - Major pathogens of animals such as *E.coli, Salmonella* and *Yersinia*
    - Opportunistic pathogens that are known occasionally to cause infection in animals.
        - These include species within the genera *Klebsiella, Enterobacter. Proteus, Serratia, Edwardsiella, Citrobacter. Morganella* and *Shigella.*
    - Uncertain significance for animals. These include species from 17 genera of this family. Eg. *Erwinia, Hafnia, Providencia*.

E. coli

To know in detail about,

- Coliforms, white scours, watery mouth, mushy-yolk disease and Hjarre's disease
- Diseases caused by *E.coli* in domestic animals
- Antigens, toxins and virulence factors of E.coli
- IMViC test
- Classification of pathogenic E.coli
- Role of Mac Conkey agar in diagnosis of Enterobacteriaceae
- General approaches used to isolate, identify and serotyping of E.coli

HABITAT

- *E. coli* is a natural inhabitant of the large intestine and lower small intestine of all mammals.
- It is usually present in large numbers in carnivores, omnivores than in herbivores.
- *E .coli* is voided in faeces and can survive in faecal particles, dust and water for weeks or months.
- The presence of *E .coli* in water samples, being tested for potability, is taken as evidence of faecal pollution.
- This genus is named after Theobald Escherich, who was first to describe the Colon bacillus under the name Bacterium coli commune (1885).

MORPHOLOGY AND CULTURAL CHARACTERISTICS

Morphology

- Gram negative, rods, non-spores, non-acid fast, occurs either in singly or in pairs.
- Usually motile with peritrichous flagella. Some strains possess a polysaccharide capsule and fimbriae.

Cultural characteristics

- It is an aerobe and facultative anaerobe, optimum temp. is 37^{o}C, good growth occurs on ordinary media.
- Colonies are large, thick, grayish white, moist, smooth and opaque.
- The 'S' forms are seen on fresh isolation.
- The 'R' forms seen on repeated subculture.
- On blood agar, many strains, especially those isolated from pathological conditions, are Beta haemolytic.
- As they are very strong lactose fermenters, the colonies on MacConkey agar are bright pink. Because of its ability to ferment sugars, it gives yellow - green colonies on Brilliant green agar (BGA) and yellow colonies on XLD agar.

- On Eosin methylene blue agar (EMB), *E.coli* colonies have a unique and characteristic metallic sheen. Click here for visual

- In nutrient broth, growth occurs as general turbidity and a heavy deposit, which disperses completely on shaking.

BIOCHEMICAL PROPERTIES AND RESISTANCE

Biochemical properties

- The four-biochemical tests- IMViC- (Indole, Methyl Red, Voges- Proskauer, Citrate utilization) are widely employed to identify *E.coli.*

- It gives IMViC, **+,+, -, -.**

- They ferment several sugars and produce acid and gas.
- H_2S negative, Urease negative, Oxidase negative and Catalase positive.

Resistance

- *E.coli* remains in the environment (water, feed, dust) for weeks.
- Some strains are more heat resistant and they will survive at 60°C for 15 min or 55°C for 60 min.
- They are more sensitive to lethal action of phenol and cresol.

ANTIGENS AND TOXINS

- The capsular antigens (K) are polysaccharides and the cell wall or somatic (O) antigens are determined by the sugar side chains on the LPS of the outer membrane.
- The Flagellar (H) and Fimbrial (F) antigens are proteins.
- Three kinds of K antigens L, A, B have been described based on the effect of heat on the agglutinability, antigenicity and antibody binding power.
- The O, H and K antigens can be used to serotype strains *of E.coli.* So far, 164 types of O antigens, 103 K antigens and 75 H antigens have been recognized.
- The antigenic pattern of a strain is recorded as the number of particular antigen it bears for eg: 0157: K85: H19.

Toxins and virulence factors of E.coli

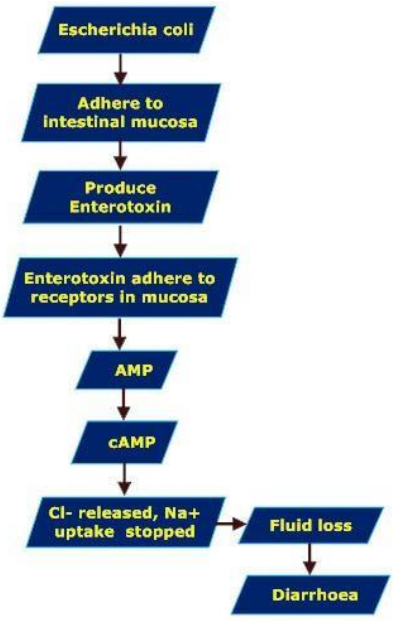
- Capsule
  - The capsular polysaccharide is antiphagocytic and also protects the cell wall from the damaging effects of complement.
- Cell wall
  - The endotoxin (lipid A) is the toxin associated with colisepticaemias and the toxaemia in coliform mastitis.
  - Lipid A also interferes with the complement components that are responsible for the attack on the outer membrane *of E.coli.*
  - Endotoxin is released when bacteria die and lyse.
  - The effects of endotoxin in the animal body include fever, leukopenia followed by leukocytosis and hyperglycemia with a subsequent fall in blood sugar and lethal shock after a latent period.
- Fimbriae/ Pili
  - Fimbrial antigens are adhesins (i.e. they confer the adhesive properties on the organism).
  - Several major specific adhesins Type I (mannose sensitive), mannose resistant K88 (F4) and K99 (F5), F6 and F 41 allow the attachment *of E.coli* to RBC's or glycoproteins on the surface of the epithelial cells of jejunum and ileum with consequent colonization.
- Enterotoxins
  - Both heat labile (LT) and heat stable (ST) enterotoxins are produced by the ETEC strains that also have the K88, K99 or other colonizing agents.
  - The LT enterotoxin is a large molecular weight protein and is antigenically related to cholera toxin, which causes stimulation of asenylate cyclase activity.
  - The ST enterotoxin is also a protein and there are two types, STa and STb. STa toxin increases the guanylate cyclase system.
- Verotoxins or shiga like toxins or Odema disease toxin
  - It inhibits protein synthesis in host cells following interaction with 60S ribosomal subunit.
  - There are two types,SLT I / VT-1- that are neutralized by shiga antitoxin, SLT 2 / VT2e - which causes odema disease in pigs and haemorrhagic colitis in man and it is not neutralized by shiga antitoxin.

    - Shiga like toxin can cause destruction of intestinal epithelial cells where there is dense adherence of the pathogenic *E.coli*.
- Haemolysins
    - *E.coli* strains produce both alpha and beta haemolysin.
    - The alpha haemolysin is a protein that damages host cell membranes and also increases the availbility of iron, which is required for invading organisms in the host.
- Siderophores
    - Siderophores – iron-binding molecules are particularlyimportant for the invasive strains.
    - In response to low level of iron, the siderophores – aerobactin and enterobactin are synthesized and released which allows the bacteria to multiply in an environment of limited concentration of free iron.
- Colicins
    - Colicins are distinctive class of proteinaceous antibiotic substances produced by strains of *E.coli*, which was described, by Gratia and Frederiq in 1946.
    - They are 40-60 Kda, plasmid encoded proteins that are released extracellularly.
    - Characteristically, they become attached to specific receptors on the surface of susceptible bacteria, causing the death of these organisms.
    - Colicin V (COLv) is found primarily among virulent bacteria.
    - It is plasmid encoded, small molecule and is released by an export mechanism.
    - The Col V plasmid also encode for virulence, increased serum survival, altered motility, changes in hydrophobicity and facilitates attachment to appropriate host cells.

PATHOGENESIS

Predisposing factors

- They are paramountimportance for establishing the disease. Young neonates, particularly one week of age, are particularly susceptible because,
    - Normal flora of the intestine is not fully established
    - Have a naive immune system
    - Receptors for the adhesions of *E.coli* (i.e. ETEC strains) are present for the first week of life only in calves and for the first 6 weeks of life in piglets.
- In addition to this, poor husbandry practices, insufficient passive immunity, change of environment and healthy grain diets in weaned pigs leads to massive colonization of the intestine by *E.coli*.



S.No	Animals	Disease
1	• Cattle • Calves less than one week old • Calves surviving colisepticaemia • Cows	• Colibacillosis (White scours) or Colisepticaemia • Joint ill • Coliform mastitis
2	• Sheep • Neonatal lambs • Ewes	• Colibacillosis and Colisepticaemia • Watery mouth • Coliform mastitis
3	• Pigs • Piglets less than one week old • Pigs about 2 weeks • Weaned pigs • Sow	• Colibacillosis and colisepticaemia, Piglet meningitis Weanling enteritis • Odema disease • Coliform mastitis, MMA(Mastitis, Metritis, Agalactia syndrome)
4	• Dogs • Neonatal pups • Bitches • Adult dogs	• Colisepticaemia (Fading puppy syndrome) • Pyometra • Urinary tract infection
5	• Poultry • Young chicks • All ages	• Omphalitis (Mushy -yolk disease) • Airsacculitis, Colibacillosis, Hjarre's disease or Coligranuloma • Swollen head syndrome in broilers

PATHOGENICITY

Classification of pathogenic *E.coli*

- Classification of enteric *E.coli* pathogens
    - *ETEC:* Enterotoxigenic *E.coli,* which has the firmbrial adhesins K88, K99 etc. the production of these colonization factors, correlates with enterotoxin production. These strains cause the majority of cases of neonatal Colibacillosis. An adhesin, termed intimin is necessary for the binding of EPEC to enterocytes.
    - EPEC: Enteropathogenic *E.coli* -Do not produce any toxins but they can cause destruction and stunting of microvilli, atrophy and shedding of enterocytes which leads to enteritis and diarrhoea.
    - *EIEC:* Enteroinvasive *E.coli*- strains adhere to cells of the distal small intestine; invade the erythrocytes and deeper layers of the intestinal mucosa. They reach the lymphatic system where there is multiplication. Several virulent factors areimportant as survival for these invasive strains, which are responsible for colisepticaemia.
    - *AEEC:* Attaching and Effacing *E.coli* strains colonise the small intestine, attach to target cells and kill them. The verotoxins destroy the microvilli. These strains have been isolated from calves and rabbits.
    - *EHEC:* Enterohaemorrhagic *E.coli*: Two types are recognized. VTEC- Verotoxigenic *E.coli*. Eg. O157:H7-which is responsible for the haemorrhagic colitis-haemolytic ureamic syndrome in humans and SLTEC- Shiga like toxin producing *E.coli*.
- Classification based on Haemolysis
    - *E.coli* may produce atleast 4 types of haemolysins- alpha , beta , gamma and enterohaemolysin.
- Classification based on plasmid
- Colicin typing
- Phage typing

Symptoms

Horse

- Newborn foals suffer from a disease known as Joint ill, navel ill or sleepy foal disease. There will be a rise in temperature, general weakness, diarrhoea, lameness and death.

Cattle (K99)

- It causes white scour or colisepticaemia in calves. In adult cases the symptoms are scouring, weakness, prostration and death within hours after initial symptoms.
- In less acute cases the calves become listless, fail to suck and develop diarrhoea.
- The faeces are grayish white in colour (hence the term white scour) with fetid odour.
- Other signs include swollen joints and pneumonia. Faeces with blood stained mucus, subnormal temperature, animal becomes comatose and dies.
- *E.coli* is also associated with acute bovine mastitis.
- The udder becomes swollen, hot and painful. Milk production falls rapidly and cease with systemic disturbances.

Pigs (Serotypes O8, O138, O141 and O147) (K88.K99)

- *E.coli* infection causes piglet diarrhoea and edema disease. Piglets 4 days old to 3 weeks of age are susceptible.
- There will be diarrhoea, listlessness. They may collapse and die.

- There may be odematous lesions in various parts of the body.
- They will show twitching of the ears and trembling staggering gait.

Poultry (O2,O78,O1)

- *E.coli* infection causes colibacillosis, airsacculitis, Hjarre's disease or coligranuloma and yolk sac infection.
- Death of some birds occurs rapidly while some birds show loss of appetite, respiratory distress, diarrhoea and weakness.
- Coligranuloma is a chronic condition characterized by granulomatous lesions in the epithelium of the intestine and other organs. In broilers it causes swollen head syndrome.

Dogs (O42)

- It causes fading puppy syndrome in newborn puppies.
- The only symptoms before death are weakness and lack of appetite.
- In older dogs it is associated with acute enteritis and pyometra in bitches.

Sheep and goats (K99)

- Similar lo that of calves. The watery mouth is characterized by severe depression, loss of appetite, profuse salivation and abdominal distension.

Lesions

Cattle

- In cases of pneumonia the lungs may show areas of congestion and necrosis.
- The spleen and mesenteric lymph nodes are enlarged and congested. Joint infections develop as synovitis.

Sheep and Goats

- Similar to that of calves.

Pigs

- The intestine shows area of congestion and the stomach is filled with clotted milk.
- In oedema disease the carcass shows edematous areas of eyelids, ears and face.
- The abdominal cavity, pleural and pericardial sac may contain clear fluid containing fibrin.

Poultry

- Characteristically there is pericarditis, perihepatitis containing a quantity of purulent exudate.
- Other lesions include inflammation of air sacs, which may contain caseous material, congested liver with gelatinous exudate and an enlarged spleen.

Dogs

- The lesions include petechial haemorrhages throughout the carcass, congested lungs and haemorrhagic gastroenteritis.

DIAGNOSIS

- Clinical signs and pathology
- Specimens to be collected
    - It includes faecal samples in case of enteric disease, tissue specimens from septicaemic cases, mastitis milk, mid-stream urine and cervical swabs from suspected cases of pyometra or metritis.
- Isolation, Identification and serotyping
    - Isolation can be done in blood agar, MacConkey agar and EMB agar.
    - The organisms can be isolated in pure culture from the small intestine.
    - In septicemic conditions *E.coli* can be recovered from the liver, spleen, kidneys and lungs.
        - Lab. techniques for detection of enterotoxins.
        - Detection of heat labile enterotoxin by using mouse adrenal cells, Chinese hamster ovary cells and vero monkey kidney cells.
        - Ligated ileal loops of rabbits are injected with bacterial culture supernatants.
        - A positive test is indicated by the accumulation of fluid in the ligated loop.
        - Detection of heat stable enterotoxins (ST) is done by injecting culture supernatants into milk filled stomach of infant mice.
        - After 4 hours the mice are sacrificed & examined for dilatation of the intestine due to fluid accumulation.
        - The enterotoxins or fimbrial antigens can also be confirmed by immunological methods or by PCR.
        - For expression of fimbrial antigens, isolates should be subcultured on minca medium.
        - Slide agglutination tests for O and H antigens are employed for serotype identification.

CONTROL AND PREVENTION

Note

- Colostral antibodies can prevent colonization of the intestine by pathogenic *E.coli*.
- Absorption of gammaglobulin from the intestine declines progressively after birth and is negligible by 36 hours.

Passive immunization

- Passive immunization can be achieved by immunizing sows with *E.coli* K88 antigen during gestation.
- This results in the production of anti K-88 antibodies in the colostrum and milk.
- This when ingested by piglets prevent the adhesive capacity of K88 antigen on the bacteria.
- In cattle the mother should not be moved to new environment shortly before calving.

Active immunization

- Three types of vaccines areavailable. They are live *E.coli* K88 antigen (oral vaccine), killed *E.coli* bacteria with K88 antigen (bacterin) and bacteria free K88 antigen (subunit vaccine).
- Vaccination of pregnant cows with purified *E.coli* K99 fimbrial or whole cell preparations, often combined with rotavirus antigen, can be used toenhance colostral protection.
- Antibiotics have been used in the prevention and treatment of colibacillosis, particularly oxytetracycline, chlortetracycline, streptomycin, ciprofloxacin, and enrofloxacin. But this has resulted in the development of resistant strains of *E.coli.*

SALMONELLA

Learning objectives

To know in detail about,

- Definition for pullorum disease and fowl typhoid
- Cultural and biohmeical characters of *Salmonella sp*
- Role of enrichment broth in isolationm of *salmonella sp*
- Antigens, toxins and antigenic variations of Salmonella
- Different classification scheme of *Salmonella sp*

HISTORY

- Salmonella has long been recognized asimportant zoonotic pathogen of worldwide economic significance in humans and animals.
- Infections of animals with various species of Salmonella sometimes result in serious disease.
- The interplay of Salmonella with its host takes a variety of forms, including remarkable host specificity, inapparent infection, recovered carriers, enteritis, septicaemia, abortion and combination of disease syndromes.
- The typhoid bacillus *Salmonella typhi* was first observed by Eberth (1880) in the mesenteric nodes and spleen of fatal cases of typhoid fever and was isolated by Gaffky (1884).
- It came to be known as Eberth – Gaffky bacillus or Eberthella typhi.
- Salmon and Smith (1885) described a bacillus, which was believed to cause hog cholera in swine.
- This bacillus, now called *Salmonella cholera suis* was the first of a series of similar organisms to be isolated from animal and man- the genus Salmonella
- Salmonellae currently comprise about 2400 serotypes, of which 50 of them are potentially pathogenic.

HABITAT

- The reservoir for salmonellae is the intestinal tract of warm blooded and cold-blooded animals.
- The majority of infected animals become sub clinical excretors resulting in contamination of water, food and the environment.
- In Poultry some serotypes, such as *Salmonella enteritidis* infect the ovaries and be transmitted through eggs.
- The undercooked egg dishes may result in human food poisoning.
- The salmonellae can survive for 9 months or more in moist soil, water, faecal particles and animal feeds, especially in blood, bone and fish meal.

MORPHOLOGY

- Salmonella are Gram-ve, non-capsulated, non-spore forming, short rods.
- They are motile by peritrichous flagella, except *S.gallinarum* and *S.pullorum*.
- Most salmonella strains possess type –1 fimbriae associated with mannose sensitive adhesive properties.
- Strains of *S.gallinarum* and *S.pullorum* form type 2 fimbriae, which are morphologically and antigenically like type 1 fimbriae but non-adhesive.

CULTURAL CHARACTERISTICS, BIOCHEMICAL PROPERTIESAND RESISTANCE

Cultural characteristics

- They are aerobic and facultative anaerobic, growing readily on simple media over a pH range of 6.8. Optimum temperature for growth is 37^{o}C.
- On nutrient agar the colonies are large, circular, low convex and smooth.
- The selective enriched media for salmonellae are tetrathionate broth, selenite broth and rappaport vasiliadis medium.
- The host adapted serotypes from pigs and poultry are more fastidious than others.
- They do not tolerate selenite broth and tetrathionate broth. In this case, Rappaport is highly suitable.
- After 24-48 hr incubation on selective broth the subculture will be made on MacConkey agar, Brilliant green agar, XLD and *Salmonella Shigella* (SS) agar.
- The majority of salmonellae, except some strains of *S.arizonae,* are non-lactose fermenters and produce pale or colorless colonies on MacConkey agar.
- Most salmonellae give an alkaline reaction in brilliant green agar and have red colonies

- On XLD medium, they produce H_2S and have red colonies with a black center (Black center with red skirt).
- On Salmonella and Shigella agar they produce colorless colonies with black center.
- The typical reaction for salmonellae in TSI (triple sugar iron) agar is an alkaline slant (red), acid butt (yellow) and superimposed H_2S (black) production ($R/Y/H2S^{+}$). Click here for visual
- The test for lysine decarboxylation is positive.

Biochemical properties

- *Salmonella* gives IMViC test -, +,-,+. They ferment maltose, mannitol, mannose and glucose and produce acid and gas. But do not ferment lactose, sucrose and salicin.
- Urease negative. Most salmonellae produce H2S except *S.cholera suis* and *S.paratyphi A*.
- *Salmonella pullorum* ferments glucose and rhamnose while *S.gallinarum* ferments dulcitiol and maltose.

Resistance

- The bacilli are destroyed at 55 ^{o}C in one hour or at 60 ^{o}C in 15mts.
- Boiling or chlorination of water and pasteurization of milk destroys the bacilli.
- Cultures may be viable for years if prevented from drying.
- They are killed within 5 minutes by mercuric chloride (1:500) or 5% phenol.

ANTIGENS AND ITS VARIATIONS

Antigens

Flagellar antigen

- It is heat labile protein when mixed with H antisera, agglutinates rapidly producing large, loose fluffy clumps.
- The H antigen is strongly immunogenic. They have the unique character of diphasic variation.

Somatic antigen

- It is a heat stable phospholipid protein polysachharide complex, which forms an integral part of the cell wall.
- It is identical with endotoxin.
- Boivin is extracted from the bacterial cell by treating with trichlor acetic acid hence, it is called as Boivin antigen.
- When mixed with O antiserum, it forms compact chalky granular clumps. The O antigen is less immunogenic than H antigen.

Vi antigen

- Many strains of *S.typhi, S.paratyphi* and *S.dublin* fail to agglutinate with O antiserum due to the presence of Vi antigen enveloping the O antigen.
- The Vi antigen is related to virulence and may be lost by serial subculture. The Vi antigen is poorly immunogenic.
- In addition to endotoxin, enterotoxins similar to heat labile entero toxin of *E.coli* and cholera toxin are produced. The cytotoxin similar to shigella cytotoxin is also produced.

Antigenic variations

- The antigens of salmonellae frequently undergo phenotypic and genotypic variations

H-O variation

- This variation is associated with loss of flagella. When Salmonella are grown in agar containing phenol (1 in 800), flagella are inhibited. This change is phenotypic and temporary.
- To attain a population of *Salmonella* rich in H antigen Craigie's tube or U tube on soft agar can be employed.

Phase variation

- Phase 1 antigen is either specific for a species or shared by a few species only. Hence termed as specific phase.
- Phase 2 antigens are widely shared and hence termed as nonspecific or group phase.
- Phase 1 antigens are designated as a, b, c...Z and after Z as Z_1, Z_2, Z_3...etc. Phase 2 is designated as 1,2...etc.
- The flagellar antigens of salmonellae occur in 1 or 2 phases.
- Strains that posses both phases are called diphasic and strains having 1 phase are called as monophasic.

V-W variation

- Fresh isolates of *S. typhi* carry a surface Vi antigen that completely masks the O antigen.
- Such bacillus does not agglutinate with O antiserum but agglutinable with Vi antiserum. This is called as V form.
- After repeated sub culturing the Vi antigen is completely lost and does not agglutinate with Vi antiserum but agglutinate with O antiserum.
- This is called as W form. Intermediate stages which agglutinate with both Vi and O antisera is called VW form.

S-R variation

- The smooth to rough variation is associated with the change in colony morphology, loss of O antigen and virulence.
- The colonies become large, rough and irregular.
- R forms occur by conversion into mutation.
- It can be prevented by maintaining the cultures by lyophilisation.

Variation in O antigens

- Changes in the structure of O antigens may be induced by lysogenisation with certain phages resulting in alteration of serotypes.

CLASSIFICATION OF SALMONELLA

O, H and Vi antigens

- Based on biochemical reactions,(Reeves, et.al., 1989), the genus Salmonella is divided into two species, *S.enterica* and *S.bongori*.
- *S.enterica* is subdivided into 6 subspecies. Enterica (I), Salamae (II), Arizonae (III a), Diarizonae (III b), Indica (IV) and Houtenae (V).
- Sub genus enterica is the largest and most important, containing all the species that commonly cause human and animal infections.
- Members of this sub genus are given a name like *S.enterica* sub sp. enterica serovar typhimurium.
- Serological classification is done by Kauffmann – white scheme. It depends on the identification by O and H antigens of the strain and agglutination
- Example

O serogroup	*	Serotype	Antigens		
			O	H	
				Phase 1	Phase II
2	A	Paratyphi A	1,2,12	a	-
4	B	Paratyphi B Typhimurium	1,**4**,5,12 1,**4**,5,12	b c	1,2 1,2
7	C	Cholera suis Paratyphi C	6,7 6,7 (W)	c c	1,5 1,5

9	D	Typhi Enteritidis Pullorum and gallinarum Dublin	**9** ,12 (W) 1,**9**,12 1,**9**,12 1,**9**,12 (Vi)	d g, m - g, p	- 1,7 - -

- Primary subdivision is into O serogroups, each of which shares a common somatic antigen.
- Where more than one O antigen is present, one of them is the major O Ag and is regarded as determining the group to which the strain shall be allocated.
- O serogroups were formerly designated by letters of the alphabet (A-Z).
- But now are indicated by numbers 1,2,3... The series of O antigens numbered O1 –67 is not continuous because some O antigens were originally assigned to bacteria that proved subsequently not to be salmonellae. Thus, only 46 O serogroups are defined by the 67 O antigens described.

PATHOGENESIS

Salmonella

- Ingestion
- Gastroenteritis form Enteric fever form
- Colonize intestine Colonize intestine
- Penetrate epithelial cells of intestinal villi Penetrate epithelial cells of intestinal villi
- Produce enterotoxins Enter blood stream
- Gastroenteritis Endotoxaemia

Abortion

- Following adherence with fimbriae to the surface of the intestinal mucousal cells, the bacteria induce ruffling of cell membranes.
- The ruffles facilitate uptake of the bacteria in membrane bound-vesicles, which often coalesce.
- The org. replicate in these cells and eventually released from the cells.
- The virulence of salmonellae relates to their ability to invade host cells and replicate in them.
- The O-antigen chains of LPS resist phagocytosis and long chains of LPS prevent complement action, which will facilitate the spread of organisms.
- The LPS is also responsible for endotoxin activity.
- Salmonellae often localize in the mucosae of the ileum, caecum and colon, and in the mesenteric lymphnodes of infected animals. Subclinical infection may persist with small numbers of salmonellae in the faeces. Latent infections, in which salmonellae are present in the gall bladder but are not excreted, also occur.
- Stress factors such as inter-current infections, transportation, overcrowding, pregnancy, extreme ambient temperature, sudden changes in rations etc may activate latent or sub clinical salmonellosis.

Diseases caused by Salmonella

Host	Salmonella serotypes	Disease

Cattle	• *S.dublin* • *S.typhimurium*	• Enteritis, septicaemia, meningitis in calves, abortion, osteomyelitis, joint ill, terminal dry gangrene in calves. • Enteritis or septicaemia
Pigs	• *S.cholera suis* • *S. typhisuis* • *S.typhimurium*	• Outbreaks clinically similar to swine fever • Chronic enteritis in young pigs • Enteritis or septicaemia
Sheep	• *S.abortus ovis* • *S.typhimurium* • *S. brandenburg*	• Abortion • Enteritis or septicaemia • Abortion
Horse	• *S.abortus equi* • *S.typhimurium*	• Abortion in mares • Enteritis or septicaemia
Poultry	• *S.pullorum* • *S.gallinarum* • *S.arizonae* • *S.enteritidis, S.typhimurium and many other serotypes*	• Pullorum disease (bacillary white diarrhoea) in chicks • Fowl typhoid in all ages. Mainly adults. • Severe enteritis and septicaemia in chicks, turkey poults (Arizona). • Fowl paratyphoid
Human	• *S.typhi* • *S.paratyphi* • *(S.paratyphi A)* • *S.schottmuelleri* • *(S.paratyphi B)* • *S.hirschfeldri* • *(S.paratyphi C)* • *S.enteritidis* • *S.typhimurium*	• Typhoid fever • Paratyphoid fever • Food poisoning

PATHOGENICITY

Pathogenesis

Cattle

- *S. typhimurium* and *S. dublin* infections in adult cattle gives rise to a rise in temperature, inappetance, a sudden drop in milk yield and diarrhoea. This is followed by dysentery.
- Pregnant animals may abort and death occurs in 2 to 3 days.

- Terminal dry gangrene and bone lesions are common in chronic infections with *S. dublin* in calves.

Horses

- The serotypes commonly affecting horses are *S. abortus equi* and *S. typhimurium*.
- Abortion occurs during the last 2 months of pregnancy or at full term.
- In newborn foals, infection results in septicaemia, weakness, diarrhoea, pyrexia, laboured breathing and death in 2-3 days.
- In some cases infections occur in the naval and joints.

Sheep and Goat

- The common serotypes isolated are *S.abortus ovis* and *S. typhimurium*.
- Abortion occurs during the last month of pregnancy.
- Death in lambs occurs during the first day after birth with diarrhoea and dysentery.

Pigs

- The serotypes commonly found are *S. cholera suis* and *S. typhimurium*.
- Pig's paratyphoid can occur as an acute, subacute and chronic disease.
- In acute form there will be rise in temperature, inappetance and discoloration of the skin.
- Bluish discoloration of the ears and snout is characteristic.
- Death occurs in 1-4 days after the onset of symptoms.
- In less acute cases there will be profuse yellowish diarrhoea, poor appetite, weakness and death in animals.

Poultry

- Diseases in poultry include infections with *S. pullorum* (Pullorum disease/Bacillary white diarrhoea) , *S. gallinarum* (Fowl typhoid) and *S. typhimurium* (Avian paratyphoid).
- In Pullorum disease the newly hatched chicks may die without any marked symptoms.
- Other symptoms include huddling near the heat source, loss of appetite, drooping of wings and head, whitish diarrhoea with pasty vent and respiratory distress.
- The majority of infected adults have lowered egg production.
- In fowl typhoid disease occurs among growers and adults with a mortality rate of 50 %.
- The symptoms include listlessness, diarrhoea with greenish coloured faeces and paleness of wattle and comb. This is followed by death.
- In avian paratyphoid newly hatched birds are susceptible and symptoms are similar to that of pullorum disease.

Lesions

Cattle

- There are areas of haemorrhagic inflammation and necrosis of the large intestine.
- The mesenteric lymph nodes are oedematous and haemorrhagic.
- Areas of necrosis occur in the liver and spleen.

Horse

- Petechial haemorrhages occur in the heart, spleen and lungs of the aborted fetuses.
- Fetal membranes are oedematous, haemorrhagic with areas of necrosis.

Sheep and Goat

- The abomasum and small intestine have areas of congestion and haemorrhage.
- Haemorrhages occur in the myocardium and kidney cortex with oema of the mesenteric lymph nodes.

Pigs

- In acute cases the mucosa of stomach, intestine, kidney cortex and myocardium reveals haemorrhagic spots.
- Mesenteric lymph nodes and spleen are enlarged and hyperaemic. There may be discoloration of the skin.

Poultry

- In Pullorum disease, the liver is yellowish in color with haemorrhagic streaks.
- In chronic cases the ovary consists of pedunculate and misshapen ovules, which is characteristic.
- In fowl typhoid the most obvious lesion includes enlarged and congested liver, which becomes dark red or brown (bile stained liver) after exposure to the atmosphere.
- There may be multiple necrotic areas through out the liver.
- In Avian paratyphoid there will be congestion and necrosis of the liver and spleen with catarrhal enteritis.

DIAGNOSIS

Specimens to be collected

- It includes faeces and blood from live animals, intestinal contents and tissue lesions from dead animals, abomasal contents from aborted fetuses and parenchymatous organs from septicaemic conditions.

Diagnosis based on

- Symptoms and lesions
- Isolation and identification of the organism - If the organism is less, initially it is enriched in peptone water and then inoculated in selective enrichment broth.
- After incubation for 48 hours they are streaked onto selective enriched media.
- The samples used are aborted fetus, feces, blood, milk, egg and visceral organs.
- Agglutination test: In horses the O agglutinin titre of 1/1000 or more is considered positive.
- Presence of agglutinin titre against H antigen indicates active infection.
- In cattle a titre of 1 in 40 against O antigen and l in 320 against H antigen is considered positive.
- In poultry a titre of l in 50 & l in 25 is indicative of active infection.
- (Note: The agglutinin titre will vary with different areas for positive cases) (Click here for visual)

- Whole blood agglutination test: 1 drop of colored or plain pullorum antigen is mixed with 1 drop of blood and allowed for 2 minutes. In positive cases there will be clumps.

- Tube agglutination test: In this test the sample serum is diluted serially to which 0.5 ml of constant amount of antigen is added and kept at 37°C in a water bath overnight.
- Agglutinations in dilutions at a titre of 1 in 40 indicates *a* positive test.

CONTROL, PREVENTION AND PUBLIC HEALTH ASPECTS

Control and prevention

- In horses, cattle, sheep and goat live vaccines give rise to solid immunity but the animal may remain as carrier throughout its life.
- Killed vaccines are safe but can stimulate only a temporary resistance.
- For cattle attenuated live culture of *S. dublin* vaccine has been developed.
- In poultry either killed or live attenuated vaccine stimulates good immune response.
- Various chemotherapeutic agents have been used to treat salmonellosis including Chloromycetin, Terramycin, neomycin, furazolidone and sulfasuxidine.

Public Health aspects

- The majority of serotypes are potential pathogens for both man and animals.
- Theimportant route of transmission is through milk, meat and egg.
- This will result in *Salmonella* food poisoning with symptoms of severe gastroenteritis.

YERSINIA

INTRODUCTION

- *Yersinia* species are non-lactose fermenters and, with the exception of Y. pestis are motile.
- Although there are more than 10 *Yersinia* species, only Yersinia pestis , Yersinia enterocolitica , Yersinia pesudoteuberculosis are pathogenic for animals and man.
- They characteristically demonstrate bipolar staining in Giemsa-stained smears from animal tissues.
- Yersinia pesudotuberculosis and Yersinia enterocolitica are found in the intestinal tracts of a wide range of wild mammals, birds and domestic animals.
- All these animals may be reservoirs of infection. Many avian species may act as amplifier hosts and may also transfer the organisms mechanically.
- They able to grow in a wide temperature range (5 to 42°C) and survive for long periods in cool wet conditions.
- In endemic areas, wild rodents areimportant reservoirs of Yersinia pestis.
- Fleas, especially *Xenopsylla cheopis*, the oriental rat flea, transmit the infection to man and other animals.
- Diseases caused by *Yersinia*

Species	Host	Disease
Yersinia enterocolitica	Pigs, other domestic animals	Enteritis (sub clinical infections)
	Ewes	Sporadic abortion
Yersinia	Guinea-pigs and other	Septicemia (Pseudotuberculosis) and

pseudotuberculosis	laboratory animals	Focal hepatic necrosis
	Sheep, goats, cattle, buffalo and pigs	Enteritis, mesenteric lymphadenitis
	Cattle, sheep, goats	Sporadic abortion
Yersinia pestis	Humans	Bubonic and pneumonic plague
	Rodents	Sylvatic plague
	Cats	Feline plague

YERSINIA PSEUDOTUBERCULOSIS

Synonym: *Pasteurella pseudotuberculosis*

Distribution

- The organisms has a world wide distribution and is particularly associated with a disease known as pseudotuberculosis in guinea pigs and to a less extent in turkeys, pigeons, rats, rabbits and horses.
- It has occasionally been isolated from sheep, goats, pigs, cattle, cats & man.

Morphology

- *Y. pseudotuberculosis* occur as Gram negative short ovoid rods.
- They are motile at 22°C and show bipolar appearance with leishman's staining.

Cultural characters

- It can grow on MacConkey agar, which is a point of differentiation between *Pasturella multocida* and *Yersinia*.
- Indole is not produced & nitrates are reduced to nitrates.

Antigens and Toxins

- Of the ten serotypes of Yersinia pseudotuberculosis, serotypes I,II, III contain the majority of pathogens.
- *Yersinia* posses a common flagellar antigen and a common somatic antigen.

Symptoms

- The acute form of the disease develops as a sudden and acute septicaemia.
- The septicaemic form is called as pseudotuberculosis.
- Deaths may be sudden and mortality may be high.

- In chronic cases there will be intermittent diarrhoea with emaciation before death.

Lesions

- The spleen and mesenteric lymph nodes are enlarged and congested.
- In chronic cases necrotic and caseous lesions in the spleen, liver and mesenteric Lymph nodes will be noticed, resembling pseudotuberculosis.

Diagnosis

- By isolation & identification of the organism from the liver, spleen, heart blood and bone marrow.
- A cold enrichment procedure may facilitate recovery of yersiniae from faeces, especially if they are present in very low numbers.
- A 5% suspension of faeces in PBS, held at 4°C for 3 weeks, is subcultured weekly on MacConkey agar.

Control and Prevention

- The organisms are susceptible to chloramphenicol and streptomycin.

Public health aspects

- *Y. pseudotuberculosis* in man produce chronic infection of the mesenteric lymph nodes and produce symptoms suggestive of an appendicitis.

YERSINIA PESTIS

- This organism causes bubonic plague or pneumonic plague in man. It is carried by rats & other rodents.
- They are Gram-negative short coccobacilli showing bipolar staining with Leishmans stain.
- It can grow aerobically and anaerobically in the presence of serum and blood.
- It grows on MacConkey agar and capsule formation is noticed at 37°C. *Y.pestis* produces a capsular antigen and a somatic antigen.
- It causes pneumonic or bubonic plague and the lesions include swollen and haemorrhagic lymphatic glands and spleen with multiple necrotic areas in the spleen and liver.
- Diagnosis is done by isolation & identification of the organism.
- Live and killed vaccines are used for prevention and plague cases are treated with oxytetracycline & other antibiotics.
- Three clinical forms of feline plague are recognized; bubonic, septicaemic and pneumonic.
- The most common form of the disease is characterized by enlarged lymphnodes (Buboes) associated with lymphatic drainage (serosanguineous fluid or pus) from the site of infection.
- Septicaemia may occur without lymphadenopathy and is potentially fatal.
- Pneumonic lesions may result from haematogenous spread.
- Cats with pneumonic lesions are potential source of human infection through aerosol generation.

YERSINIA ENTEROCOLITICA

- *Y. enterocolitica* has been identified from pigs, horse, ox, sheep and goat, dogs, cats, rodents etc. 57 O-factors, 6 K -antigens and 19 H - antigens have been identified.
- Based on this there are five biotypes and more than 50 serotypes have been identified.
- Somatic antigens 2,3,5,8 and 9 are present in isolates from clinical infections caused by this species.
- Serotype O:9 is of particularimportance because it shares common antigens with *Brucella* species and it may induce false-positive reactions in brucella agglutination tests.
- They produce either generalized infections or enteritis.
- It can grow on blood agar and the optimum temperature for growth is 28°C.
- A preceding cold enrichment improves the isolation rate.
- It produces gastroenteritis in human infants and terminal ileitis, mesenteric lymphadenitis and pseudo appendicitis in adolescents.
- The pig is the natural reservoir for *Yersinia enterocolitica* serotype O3 biotype 4, which is animportant pathogen in humans

SHIGELLA

- Four species (*S. boydii, S. dysenteriae, S. flexneri and S. sonnei*) are etiological agents of diarrhoea and dysentery in man and subhuman primates.
- They are Gram –ve, non-motile, rod shaped organisms found in the intestinal flora of animals.
- *Shigella* are facultatively anaerobic and grows on blood agar and MacConkey agar at 37°C without haemolysis.
- *Shigella* produces an enterotoxin which acts on the epithelial lining of the terminal illeum and large intestine, producing bloody mucus stained stool.

CITROBACTER

- *Citrobacter freundi* forms part of the normal intestinal flora of domestic animals and are also found in soil and water.
- They are Gram negative, facultatively anaerobic, motile, rod shaped organisms, which grows on blood agar and MacConkey agar at 37°*C* producing no haemolysis.
- In domestic animals, *Citrobacter* are opportunistic pathogens in a variety of extra intestinal infections.

EDWARDSIELLA

- *Edwardsiella tarda* appears to have a worldwide distribution and has been isolated from all classes of vertebrates, fish, amphibia, reptiles, birds and mammals.
- They are Gram negative, motile rods. They are facultatively anaerobic and grow on blood agar and MacConkey agar at 37°C.
- They are non-haemolytic and lactose negative organisms.
- *E. tarda* has been implicated as the cause of diarrhoea,wound infections and sepsis in animals and humans.

ENTEROBACTER

- *E. cloacae* and *E.agglomerans* are the most commonly isolated species.

- They inhabit mainly the environment and they are also found in the digestive tract of animals.
- They are Gram negative, motile rod shaped organisms.
- They are facultatively anaerobic and grow on blood agar and MacConkey agar at 37°C.
- In domestic animals, *Enterobacter* species are opportunistic pathogens in a variety of extra intestinal infections.
- *Enterobacter* species is attributed to abortion and infection of genital tract in horses, abortion and mastitis in cattle and agalactia syndrome in pigs.

KLEBSIELLA

Klebsiella (Friedlanders bacillus)

- Kelbsiella species occur naturally in soil and water and are normal intestinal flora of domestic animals.
- They are non-motile, encapsulated rods arranged singly, in pairs or short chains.
- The Klebsiella are non haemolytic on blood agar and lactose positive on MacConkey agar.
- *Klebsiella pneumoniae* has O antigen (11 types) and K antigen (70 types).
- In horses Klebsiella causes inflammation of the genital mucosa and abortion and generalized infections of foals.
- In cattle it causes mastitis, generalized infections and enteritis of calves.
- In pigs it causes atrophic rhinitis and in poultry it causes air sac and yolk sac infections.
- *K. pneumoniae* and *K. oxytoca* are the most commonly isolated species from domestic animals.

MORGANELLA

- M. morganii is the normal intestinal flora of domestic animals.
- They are Gram negative, motile rod shaped organisms.
- They are facultatively anaerobic and grow on blood agar and MacConkey agar at 37° C.
- In domestic animals, *M. morganii* is an opportunistic pathogen and is most frequently isolated from wound and urinary tract infections.

PROTEUS

- *Proteus mirabilis* and *P.vulgaris* are normal intestinal *flora* of domestic animals and also are found in soil and polluted water.
- *Proteus* is Gram-negative rod shaped organism but often show pleomorphism.
- All strains are normally motile with peritrichous flagella and majority produce fimbriae. Spores and capsules are not produced.
- These organisms are aerobic and facultatively anaerobic and growth takes place at 37°C growth on solid media shows the feature of swarming.
- All species are positive to the phenylpyruvic acid. They are responsible for endometritis, gastroenteritis and arthritis.
- In pigs it causes diarrhoea and urinary tract infections.
- In dogs and cats it causes urinary tract infections, cystitis, otits externa and sepsis. **Click here for visual**

PROVIDENCIA

Domain	Bacteria
Phylum	*Proteobacteria*
Class	*Gamma proteobacteria*

- *Providencia* found in the normal animals.
- They are Gram- peritrichous flagella.
- They are facultatively blood agar and produces no negative.
- *P.rettgeri* is frequently urinary infections.

rettgeri and *P.stuartii* are intestinal flora of domestic

negative rods and motile by

anaerobic and growth on MacConkey agar at 37°C haemolysis and is lactose

isolated in pure culture from

SERRATIA

- *S. liquefaciens* and *S. marcescens* form part of normal intestinal flora of domestic animals and also occur in soil and water.
- They are Gram-negative rods and are motile by peritrichous flagella.
- They are facultatively anaerobic. On blood agar the colonies are white, pink or red incolor.
- *S. marcescens* produces a deep red pigment and on MacConkey agar at 37°C produce lactose positive colonies.

S. marcescens are isolated from conjunctivitis, pneumonia, septicaemia and endometritis in horses and in cases of abortion and mastitis in cattle.