

Family *Enterobacteriaceae*

Enterobacteriaceae is the largest family of the clinically important Gram-negative bacilli. It contains more than 51 genera and about 238 species. They can be grouped into three categories; pathogenic group (*Salmonella*, *E. coli*, and *Yersinia* spp.), opportunistic pathogens (*Klebsiella*, *Proteus*, *Citrobacter*, and *Enterobacter*) and non-pathogenic group.

General characters of *Enterobacteriaceae*:-

- 1) Enteric bacilli; many of its members present in the intestine of animals and human as a part of intestinal flora (from hence name of the family; *Enterobacteriaceae* was derived), so they can tolerate bile which is used as a selective substance present in culture media used for their isolation as MacConkey agar.
- 2) Gram-negative rods, non-spore forming and most of them are motile via peritrichous flagella (*Shigella* and *Klebsiella*, *S. Pullorum* and *S. Gallinarum* are non-motile).
Yersinia are non-motile at 37° C but several species are motile at 25-29°C.
- 3) They are aerobic or facultative anaerobes and grow optimally at 37°C.
- 4) All of them
 - a) **Oxidase negative**, this test differentiates *Enterobacteriaceae* from the other gram-negative bacilli.
 - b) Ferment glucose with acid production.
 - c) Reduce nitrate to nitrite.
 - d) Catalase positive.
- 5) According to lactose fermentation, *Enterobacteriaceae* can be classified into two main groups:
 - a) **Lactose fermenters** include *Escherichia*, *Klebsiella*, *Citrobacter* and *Enterobacter*. They produce pink colonies on MacConkey agar (used to isolate and differentiate *Enterobacteriaceae* members). Term coliforms were previously used to describe lactose fermenters.
 - b) **Lactose non-fermenters** include *Salmonella*, *shigella*, *Yersinia* and *proteus*. They produce pale yellow colonies on MacConkey agar.
- 6) *Enterobacteriaceae* have fimbriae and complex cell wall. Its lipopolysaccharide consists of three components; somatic O polysaccharide, central core polysaccharide and lipid A.
- 7) **Antigenic structure**
 - a) **Somatic (O) antigen**; O-specific polysaccharide of **LPS**, heat stable antigen, used for typing.
 - b) **Flagellar (H) antigen**; flagellar proteins of motile species, heat labile antigen, used for typing.
 - c) **Capsular (K) antigen**; capsular polysaccharide in capsulated species, heat labile antigen. It is heavy in *Klebsiella* (mucoid colonies).

Since many of *Enterobacteriaceae* members present as a part of intestinal flora in animals and human

- a) Stool samples must be inoculated into enrichment media as tetrathionate broth or selenite F broth then subcultured on solid media.
- b) Stool samples must be cultivated on highly selective media along with MacConkey agar.
- c) Direct smear from stool/faeces samples has no value in diagnosis of gastrointestinal diseases caused by their members.

Identification and differentiation of *Enterobacteriaceae* members:-

1) Morphology:

Gram-negative rods, non-spore forming and motile (*Shigella* and *Klebsiella*, *S. pullorum* and *S. gallinarum* are non-motile).

Yersinia are non-motile at 37° C but several species are motile at 25-29°C.

2) Growth on MacConkey agar:

a) **Lactose fermenters** (*E. coli*, *Klebsiella*, *Citrobacter* and *Enterobacter*) produce pink colonies.

b) **Lactose non-fermenters** (*Salmonella*, *shigella*, *Yersinia* and *proteus*) produce pale yellow colonies.

3) Growth on selective media:

a) **On EMB**, *E. coli* produces dark colonies with green metallic sheen while *Klebsiella* produces pink mucoid colonies.

b) **On Bismuth sulphate agar**, *Salmonella* produces brown to black colonies.

4) Biochemical reactions:

- a. Oxidase negative.
- b. Ferment glucose with acid production.
- c. Reduce nitrate to nitrite.
- d. Catalase positive.

Bacteria	IMVC test	Reaction on TSI	Urease	H ₂ S
<i>E. coli</i>	+ + - -	Yellow (Acidic) butt and slant	Negative	Negative
<i>Klebsiella</i>	- - + +	Yellow (Acidic) butt and slant	Positive	Negative
<i>Salmonella</i>	- + - +	Yellow (Acidic) butt and red slant (Alkaline)	Negative	Positive in some serotypes
<i>Proteus</i>		Black (Acidic) butt and red slant (Alkaline)	Positive	Positive

5) Others:

On ordinary media or blood agar, *proteus* produces characteristic swarming appearance while on MacConkeys agar media, swarming appearance is inhibited by bile salts.

Escherichia coli

Only some strains of *E. coli* are pathogenic while most of them are harmless and present as a part of the normal flora in the intestine and can benefit their hosts through synthesis of vitamin K and prevention establishment of the pathogenic bacteria in the intestine but these strains may cause opportunistic infections in extra intestinal locations.

E. coli together with *Enterococcus faecalis* and *Cl. Perfringens* are used as indicators of faecal pollution of water and food contamination.

Morphology:

- Gram negative rods, non spore-forming.
- Most strains are motile with peritrichous flagella and fimbriated.
- Some strains produce capsule and is often mucoid

Culture characteristics:

- Facultative anaerobes and grow optimally at 37°C.
- On MacConkeys agar media, it produces pink colonies.
- On EMB, it produces dark colonies with green metallic sheen.

Biochemical reaction:

- Oxidase negative, catalase positive and reduce nitrate to nitrite.
- Ferment glucose, lactose, maltose and sacrose with production of acid. On TSI, it produces yellow (acidic) butt and slant.
- Urease negative.
- **IMVC Test:** +Ve +Ve -Ve -Ve

Serotypes of *E. coli*:

- *E. coli* is serotyped on the basis of Somatic (O), Flagellar (H) and Capsular (K) antigens and each serotype is described by numbers of these antigens Ex. O139:K82:H2.
- At the present 170 O antigens, 56 H flagellar and 80 K antigens have been described.

Diseases caused by *E. coli*:-

- 1) **Mastitis.**
- 2) **Urogenital tract infections.** Uropathogenic *E. coli* is the most common cause of urinary tract infection (cystitis and pyelonephritis).
- 3) **White scours or colibacillosis** in young calves. It is characterized by grayish white diarrhea, septicaemia and death.

- 4) **Joint ill or navel ill** in foals. It is caused by *E. coli*, *Strep. equi* and *Shigella equirulis*.
- 5) **CCRD (complicated chronic respiratory disease)** in chicken. Chronic respiratory disease is caused by *Mycoplasma gallisepticum*.
- 6) **Coli-granuloma or Hijarre's disease** in poultry. It is characterized by granulomatous lesions in wall of GIT and liver.
- 7) **Different stains of Enteric *E. coli*** causes diarrhea by different mechanisms
 - a) **Enteropathogenic *E. coli* (EPEC)**; affects human, rabbits, dogs, cats and horses. They adhere tightly to the intestinal epithelium by intimin destructing the microvilli and cupping the cells and subsequently preventing their normal functions of absorption and secretion resulting in diarrhea.
 - b) **Enterotoxigenic *E. coli* (ETEC)**; affects human and animals. They adhere to the intestinal epithelium by pili and produce two enterotoxins (LT and ST) stimulate fluid and electrolytes secretion into the intestine and prevents their absorption resulting in watery diarrhea.
 - c) **Enteroinvasive *E. coli* (EIEC)**; affects human. They penetrate, invade and destruct the intestinal epithelium resulting in profuse dysentery and high fever (identical to Shigellosis).
 - d) **Enterohaemorrhagic *E. coli* (EHEC)**; the most famous strain is O157:H7. They affect human, cattle and goats. They produce verotoxin/shiga toxin cause necrosis for the enterocytes and vascular cells resulting in bloody diarrhea (no fever), in some patients may end up with the life threatening haemolytic uraemic syndrome (haemolytic anaemia, thrombocytopenia and acute renal failure).
 - e) **Entereroaggregative *E. coli* (EAggEC)**; affect human. They adhere to mucosa in patches and produce a ST-like toxin and a haemolysin resulting in persistent watery diarrhea (no fever).
- 8) In human, *E. coli* is one of the most common causes of septicemia and meningitis among neonates. It is acquired in the birth canal before or during delivery.
- 9) In human, *E. coli* causes pneumonia, septicemia and endotoxic shock following any *E. coli* infection especially in the immunocompromised hosts or neonates.
- 10) *E coli* is major nosocomial pathogen.

Virulence factors of *E. coli*:

- **Pili**; mediates adherence of bacteria to the mucosal surfaces.
- **Capsular polysaccharide** (in capsulated strains); antiphagocytic and resists the complement.
- **Lipopolysaccharides (LPS)**; Endotoxin (lipid A) responsible for signs of the endotoxic shock associated with *E.coli* infections which may lead to death.

- **Heat stable (ST) and heat labile (LT) enterotoxins** produced by Enterotoxigenic *E. coli* (ETEC); they stimulate fluid & electrolytes secretion into the intestine and prevent their absorption resulting in watery diarrhea.
- **Verotoxin or shiga toxin** produced by Enterohaemorrhagic *E. coli*; they cause necrosis for the enterocytes and vascular cells (haemorrhagic colitis and haemolytic uraemic syndrome).
- **Haemolysins** produced by some strains of *E. coli*.

Laboratory diagnosis of diseases caused by *E. coli*:

- 1) **Sampling:** According to the infection. Stool
- 2) **Microscopical examination** Stool
- 3) **Isolation and identification.**
- 4) **Serotyping** using slide agglutination test.
- 5) **ELISA** for detection of toxins.
- 6) **PCR** for detection of toxin genes.

Genus *Salmonella*

Morphology:

Gram negative rods, non spore-forming, motile with peritrichous flagella except *S. Pullorum* and *S. Gallinarum*

Culture characteristics:

- Facultative anaerobes and grow optimally at 37°C.
- On MacConkeys agar media, it produces pale yellow colonies.
- On Bismuth sulphate agar, it produces brown to black colonies.
- Can be isolated on other media as *Salmonella Shigella* (SS) agar, Brilliant Green (BG) and Xylose-lysine Deoxycholate (XLD) agar.

Biochemical reaction:

- Oxidase negative, catalase positive and reduce nitrate to nitrite.
- Do not ferment lactose while ferment glucose, maltose and mannitol with production of acid and gas. On TSI, it produces yellow (acidic) butt and red slant (alkaline). Some serotypes produce black butt due to production of H₂S.
- Urease negative.
- Some serotypes produce H₂S.
- **IMVC Test:** -Ve +Ve -Ve +Ve

Classification of *Salmonella*:

1) Kauffman-white classification (traditional classification):

Based on somatic (O) and flagellar (H) antigens, there are about 2500 serotypes of *Salmonella*.

According to somatic (O) antigen, *Salmonella* can be classified to serogroups (A-G). The somatic (O) antigens are given Arabic numbers.

The flagellar (H) antigens are detected in two antigenic forms known as phase I and phase II. Phase I is the specific and the phase-I H antigens are designated by small alphabetic letter a – z while phase II is non-specific antigen and the phase-II H antigens are designated by Arabic numbers (1-12).

Serotype	Serogroup	Somatic (O) antigens	Flagellar (H) antigens	
			Phase 1	Phase 2
<i>Salmonella Typhimurium</i>	B	1,4, 5, 12	i	1,2
<i>Salmonella Enteritidis</i>	D ₁	1,9, 12	g, m	1,7
<i>Salmonella Dublin</i>	D ₁	1,9, 12, [Vi]	G,P	-
<i>Salmonella Gallinarum</i>	D ₁	1,9, 12	-	-
<i>Salmonella Pullorum</i>	D ₁	9, 12	-	-

2) DNA-DNA hybridization studies classification (Advanced classification):

DNA-DNA hybridization studies demonstrated that genus *Salmonella* can be divided into two species; *Salmonella enterica* and *Salmonella bongori*.

a) *Salmonella enterica* is divided into six subspecies; Enterica, Arizona, Diarizona, Indica, Houtenae and Salamae. It contains most *Salmonella* serotypes (2448 serotypes). Subspecies enterica contains the most important serotypes.

b) *Salmonella bongori* contains low number of serotypes.

Diseases caused by *Salmonella* serotypes:-

- 1) *S. Pullorum* causes **Pullorum disease** in chicks up to 3 weeks of age. Pullorum disease is characterized by white diarrhea, omphalitis and sudden death with mortality rate 95- 100 %. *S. Pullorum* is transmitted from the symptomless carrier where the organism localized in ovaries and infected hatcheries.
- 2) *S. Gallinarum* causes **Fowl typhoid** in adult birds. **Fowl typhoid** is characterized with greenish or yellow diarrhea and death with mortality rate 50 %.
- 3) *S. Arizona* causes **Arizona** (enteritis, arthritis and meningitis) in Turkey.
- 4) *S. Dublin*, *S. Typhimurium*, *S. Newport*, and *S. Abortus* causes **Salmonellosis** in young calves. It is characterized by diarrhea, septicaemia and death.
- 5) *S. Dublin* and *S. Typhimurium* causes **Enteritis** in sheep and goat.
- 6) *S. Abortus* causes **abortion** in cows, sheep, goat and equines.
- 7) *S. Typhi*, *S. Paratyphi A* and *S. Paratyphi B* causes **typhoid** and **paratyphoid (Enteric fever)** in Human.
- 8) Many *Salmonella* serotypes as *Salmonella Typhimurium* and *Salmonella Enteritidis* cause **Salmonella food poisoning** or **enterocolitis** in Human.

Laboratory diagnosis of diseases caused by *Salmonella* serotypes:

- 1) **Sampling:** According to the infection Stool
- 2) **Microscopical examination** Stool
- 3) **Isolation and identification**
- 4) **Serological identification of the isolates** by slide or tube agglutination test, LISA or IFT.
- 5) **PCR.**
- 6) **Phage typing: for epidemiological studies.**

Widal test is used for diagnosis of Enteric fever.

Genus *Proteus*

Members of this genus are widely distributed in the nature. It includes two important species; *Pr. vulgaris* and *Pr. mirabilis*.

Morphology:

Gram negative pleomorphic rods, non-spore forming and highly motile.

Culture characteristics:

- Facultative anaerobes.
- They can grow on ordinary media and blood agar showing **swarming appearance** due to their high motility.
- On MacConkey's agar media, it produces pale yellow colonies while the swarming appearance is inhibited by bile salts.

Biochemical reaction:

- Oxidase negative, catalase positive and reduce nitrate to nitrite.
- Do not ferment lactose while ferment glucose with production of acid and gas. On TSI, it produces black butt (H₂S) and red slant (alkaline).
- Urease positive. Differentiates them from *Salmonella* and *Shigella*.
- Produce H₂S.

Diseases caused by *Proteus* species:-

Opportunistic pathogens frequently isolated from urinary tract infections in Human, dogs and cats. Also, it causes wound infections in animals and human.

Genus *Yersinia*

Rodents are the natural reservoirs of *Yersiniae* and less frequently the other mammals serve as a host for them.

Morphology:

- Gram negative short bacilli, non-spore forming.

- Non-motile at 37° C but several species are motile at 25-29°C.
- With Geimsa stain, it has **safety pin appearance (bipolar staining)**.

Culture characteristics:

- Facultative anaerobes.
- Grow at wide range of temperature 25-37°C.
- On MacConkeys agar media, it produces pale yellow colonies.
- For improvement isolation of *Yersinia* from stool samples, Cold enrichment is made by making 5 % suspension of stool in phosphate buffer saline and its holding at 4°C for 3 weeks with subculturing weekly on MacConkey agar.

Biochemical reaction:

- Oxidase negative, catalase positive and reduce nitrate to nitrite.
- Do not ferment lactose while ferment glucose.
- Urease negative.

Important *Yersinia* species and diseases caused by them:-

- 1) *Y. ruckeri* causes **Enteric Redmouth diseases** in fish. One of the septicemic diseases affecting marine water and freshwater fishes.
- 2) *Y. pestis* causes **plague (Black death)** in human and cats. Transmitted from the rodents by fleas and it has three forms; Bubonic, Pneumonic and Septicemic. *Y. pestis* is used as biological warfare agent.
- 3) *Y. enterocolitica* causes yersiniosis food poisoning in human.
- 4) *Y. pseudotuberculosis* is primarily an animal pathogen cause UTI and fatal septicemia in animals. Occasionally affects human and causes fever and acute mesenteric lymphadenitis.

Klebsiella and ***Citrobacter*** are saprophytes in soil and water.

- 1) *Klebsiella pneumoniae* (normal inhabitant in the respiratory tract of human) causes a small proportion of bacterial pneumonia and occasionally causes urinary tract infection.
- 2) *Klebsiella ozaenae*, *K. rhinoscleromatis* and *K. oxytoca* are isolated from UTI in cattle, chronic mastitis, peritonitis and even septicaemia.

Shigellae produce bacillary dysentery in human.

Family *Mycoplasmataceae*

(Myces, a fungus; plasma, a form)

Mycoplasmas are the smallest prokaryotes (50-300 nm). They lack the cell wall and consequently they are extremely pleomorphic, stained poorly with Gram stain and resistant to cell wall inhibitors (β -lactam antibiotics).

Also, they are the only bacteria that contain sterols in their cell membrane, these sterols are not synthesized by the organism but acquired from the tissue or media where they grow (i.e. require sterols).

Morphology:

Small (50- 300 nm) pleomorphic microorganisms; greatly varying from coccoid bodies to branched filaments.

They are Gram-negative but poorly stained with Gram stain. They are usually non motile.

Culture and biochemical characteristics:

Mycoplasmas are fastidious microorganisms, require an enriched media for growth as PPLO agar (**pleuropneumonia like organisms** agar, **old name of Mycoplasmas**), the media is supplemented with serum (source for cholesterol) and yeast extract (source for DNA and NAD). Also, Penicillin, thallous acetate and Amphotericin B are added to the media to inhibit growth of Gram-positive bacteria, Gram-negative bacteria and fungi respectively.

They are facultative anaerobes but they grow better in an atmosphere containing 5-10% CO₂. Their growth is slow requiring 2 to 7 days at 35- 37 °C.

On PPLO, Mycoplasmas produce round micro colonies (0.2-0.6mm) with a dense opaque center (embedded in the agar) and a translucent peripheral zone resembles fried egg (**Fried-egg appearance**).

Biochemical tests used for identification of Mycoplasmas includes fermentation of glucose, hydrolysis of arginine, reduction of tetrazolium, liquefaction of coagulated serum and hydrolysis of casein.

Important *Mycoplasma* species and diseases caused by them:-

- ▶ *M. mycoides* subsp. *mycoides* causes **contagious bovine pleuropneumonia (CBPP)** in cattle. It is a highly contagious disease characterized by pneumonia and pleurisy in adults and arthritis in calves.
- ▶ *M. bovis* causes **mastitis, arthritis and pneumonia** in cattle.
- ▶ *M. mycoides* subsp. *capri* causes **pneumonia, arthritis and septicaemia** in sheep and goat
- ▶ *M. agalactia* causes **mastitis** in sheep and goat.
- ▶ *M. gallisepticum* cause **chronic respiratory disease (CRD)** and **infectious sinusitis** in chickens and turkeys. **Complicated chronic respiratory disease (CCRD)** occurs where the birds concurrently infected with *E. coli*, Newcastle disease virus, Infectious Bronchitis virus, or other pathogens.
- ▶ *M. meleagridis* causes **air sacculitis** in turkeys.
- ▶ *M. synovitis* causes **synovitis** in chickens and turkeys.
- ▶ *M. pneumoniae* causes **atypical pneumonia** in human.

Laboratory diagnosis:

1) **Sampling** → According to disease

The sample is frozen for isolation of the MOs.

2) **Isolation and identification of causative agent**

3) **Identification of *Mycoplasma* strain by growth inhibition test**

This made by cultivation of *Mycoplasma* isolate on *Mycoplasma* media as PPLO then placing filter paper discs soaked with specific antisera against different *Mycoplasma* strains on surface of the inoculated media and its incubation of the inoculated media under the previously discussed conditions then the inspection macroscopically for presence zone of inhibition around these discs. Result is indicated by formation of inhibition zone around the disc containing the specific antiserum against the isolate.

4) Digitonin sensitivity test for differentiation of *Mycoplasma* from *Acholeplasma*

This made by cultivation of the isolate on *Mycoplasma* media as PPLO then placing filter paper disc soaked with digitonin on surface of the inoculated media and its incubation under the previously discussed conditions then the inspection macroscopically for presence zone of inhibition around the disc. If inhibition zone appears around the disk, the isolate is *Mycoplasma* where it is digitonin sensitive while if there is no inhibition zone around the disk, the isolate is *Acholeplasma* where it is digitonin resistant.

5) Serological tests for

- **Detection of Ag** by IFT using specific antisera.
- **Detection of Abs** against *Mycoplasma* by ELISA, CFT and agglutination test.

6) PCR.

Family *Pasteurellaceae*

It is a family of Gram-negative coccobacilli. It includes five genera; *Pasteurella*, *Mannheimia*, *Haemophilus*, *Actinobacillus* and *Ionopipinella*.

Pasteurella* and *Mannheimia

Morphology:

Gram-negative coccobacilli, non-motile and non-spore forming. In blood smears from the infected animals stained by Giemsa or Leishman, large number of bipolar bacilli appear (**bipolarity**).

Culture characteristics:

- Facultatively anaerobic and grow optimally at 37°C.
- Can grow on ordinary media but their growth is enhanced with addition of serum or blood.
- On blood agar, the colonies are round, greyish, shiny, mucoid, haemolytic and odourless in case of *M. haemolytica* while non haemolytic and has characteristic sweetish odour in case of *P. multocida*
- On MacConkey agar, *M. haemolytica* grow as pin point red colonies while *P. multocida* don't grow on MacConkey.

Biochemical characteristics:

- Oxidase positive and reduces nitrate to nitrite.
- Ferments glucose with acid production.

Serotyping:

- According to the differences in the capsular polysaccharides, *P. multocida* is subdivided into five serogroups; A, B, D, E, F. Also, *P. multocida* is subdivided into 16 somatic types according to the differences in cell wall lipopolysaccharides.
- *M. haemolytica* is subdivided into 17 somatic types.

Pasteurella and Mannheimia species of veterinary importance

<i>Pasteurella</i>	Host	Disease conditions
<i>P. multocida</i> Type A	Cattle	Shipping fever, enzootic pneumonia complex in calves
	Sheep	Pneumonia & mastitis
	Pigs	Atrophic rhinitis & pneumonia
	Rabbits	snuffles
<i>P. multocida</i> Type B	Cattle & buffaloes	Haemorrhagic septicemia (Asia)
<i>P. multocida</i> Type E	Cattle & buffaloes	Haemorrhagic septicemia (Africa)
<i>M. haemolytica</i> biotype A(<i>P. haemolytica</i>)	Cattle	Shipping fever
	Sheep	Septicemia
<i>M. haemolytica</i> biotype T(<i>P. trehalose</i>)	Sheep	Septicemia

Laboratory diagnosis:

- 1) Sampling: According to the infection
- 2) Microscopical examination.
- 3) Isolation and identification.
- 4) PCR.

Genus *Haemophilus*
(Blood loving bacteria)

Morphology:

Gram-negative coccobacilli, non-motile, non-spore forming and non-capsulated except *H. influenza* encapsulated with polysaccharide capsule.

Culture characteristics:

- Facultatively anaerobic but grow better in presence of 5-10% CO₂.
- Fastidious bacteria, their growth requires blood and blood products (Bacterial Vitamins); **factor X and/or factor V**

- a) **Factor X (hematin)**; necessary for synthesis of enzymes involved in the respiration. It present in blood.
- b) **Factor V (NAD nicotineamide adenine dinucleotide)**; necessary for oxidation – reduction process in the growing bacteria. It present inside RBCs, so it can be provided for *Haemophilus spp.* by
 - Media contain lysed RBCs (**chocolate agar**, Fildes agar, Levinthal's agar)
 - Media contain RBCs lysed by haemolytic bacteria as *Staph. aureus* (**Staph. streak technique**). In this technique, Satellitism phenomenon is observed. In Satellitism phenomenon certain bacterial species grow much better in the immediate vicinity of colonies of other unrelated species owing to providing of an essential metabolite by the latter species.
- It produces grayish, translucent smooth - low convex colonies.
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Haemophilus Species	Growth factor required	Catalase Test	Oxidase test	Utilization of		
				sucrose	Lactose	mannitol
<i>H. somnus</i>	None	-	+	-	-	+
<i>H. paragallinarum</i>	Factor V	-	-	+	-	+

Haemophilus species and diseases caused by them:

- a) *H. paragallinarum* (3 serovars; A, B and C) causes infectious coryza in chicken and turkey. It is characterized by inflammation of the upper respiratory tract, especially para nasal sinuses (Facial swelling).
- b) *H. influenza* causes meningitis, arthritis and pneumonia in human.
- c) *H. somnus* causes Enzootic calf pneumonia, septicemia, meningoencephalitis, abortion, bronchopneumonia and mastitis in cattle.
- d) *H. somnus* ovine strain causes epididymitis in rams, mastitis in ewes and septicemia, arthritis, meningitis and pneumonia in lambs.

Laboratory diagnosis of infectious coryza:

- 1) **Sampling:** ocular & nasal discharges.
- 2) **Microscopic examination**
- 3) **Isolation and identification**
- 4) **Identification of the isolated colonies using (*Staph.* streak technique).**
- 5) **Serology tests as HI, IF.**
- 6) **A PCR.**

Genus *Actinobacillus*

Morphology:

Gram-negative coccobacilli, sometimes occurring in long chains or unpointed filaments, non-motile and non-spore forming.

Culture characteristics:

- Facultatively anaerobic.
- Can grow on ordinary media and MacConkey agar media. On ordinary media, it produces small circular bluish-grey, translucent colonies with entire edge.
- Ferment sugars with production of acids and reduce nitrates to nitrite.

Diseases caused by members of Genus *Actinobacillus*:

Actinobacillus lignieresii causes **wooden tongue/ actinobacillosis** in cattle (mainly) and Sheep. This bacterium is a part of the normal bacterial flora of the upper digestive tract. It invades mouth cavity tissue through breaks, abrasions or wounds.

In this disease, tongue becoming hard, swollen, protrudes between the lips and painful. As the disease becomes chronic, the tongue becomes hard, shrunken and immobile (**wooden tongue**). The regional lymph nodes become swelled and may ulcerated discharging milky white or slightly green pus containing the characteristic greyish granules. These abscesses may present internally in the lung and may be confused with bovine tuberculosis.

Laboratory diagnosis of wooden tongue/ actinobacillosis:

- **Sample:** pus from the lesion
- **Microscopic examination**
- **Isolation & identification**

Differential diagnosis:

- a) Lumpy jaw; affects the bones.
- b) Tuberculosis of internal organs.
- c) Grass seed abscesses.
- d) Foreign bodies in the mouth.

Genus *Campylobacter*

(Curved bacteria, originally called *Vibrio*)

They are found in the reproductive and alimentary tracts of man & animals.

***Campylobacter* species and diseases caused by them:**

- a) *C. fetus* var (subspecies) *fetus* causes Epizootic abortion in sheep and goat near the end of gestation (placentitis and abortion).
- b) *C. fetus* var (subspecies) *venerealis* causes endometritis and salpingitis (infertility) in cows (Bovine genital campylobacteriosis). This species localized in the prepuce of bull and infects cows through coitus or artificial insemination.
- c) *C. coli* causes enterocolitis in pigs.

d) *C. jejuni* causes enterohepatitis in chicken and enteritis in dogs and human.

- ▶ *H. pylori* is found in the stomach of 30 to 50% of adults in the developed countries and it is almost universal in developing countries. It is the most common cause of gastritis, gastric and duodenal ulcers in addition gastric adenocarcinoma.
- ▶ *V. cholerae* causes cholera.
- ▶ *V. anguillarum* causes **Red Pest of Eel**.
- ▶ *V. parahemolyticum* causes septicaemia in fishes.
- ▶ *V. parahaemolyticus* cause food poisoning following ingestion of raw or inadequately cooked seafood.

Morphology:

- Short Gram-negative curved rods.
- Highly motile with single polar flagellum with characteristic cork screw like motion under dark field or phase contrast microscope. The two daughter cells often remain attached at their ends and appear as wings of the gull.

Culture characteristics:

- For proper isolation, the samples should be cultured immediately or held in transport media as Clark's media where *Campylobacter* species are sensitive. Also, it is preferred to filter the sample before the inoculation to exclude other bacteria.
- Isolated on enriched media as skirrow's media and incubated in an atmosphere of 10-20% CO₂ and 5% or less O₂ (microaerophilic condition).
- On skirrow's media, produce small grayish colonies.

Biochemical characteristics:

- Catalase, oxidase & hippurate hydrolysis → positive.
- Nitrate reduction test → negative except *C. coli*.
- Don't ferment sugars giving red slant and red butt on TSI except *C. coli* that produce H₂S on TSI.

Laboratory diagnosis of diseases caused by *Campylobacter*:

- 1) **Sampling** → Semen, stomach content of aborted fetus, placenta, stool.....
- 2) **Isolation and identification of causative agent.**
- 3) **Serological tests:** ELISA for detection of Abs against bacteria.
- 4) **PCR.**

Order Spirochaetales

(Spirochaetes = coiled hair)

Classification:

Order Spirochaetales includes two families; *Spirochaetaceae* and *Leptospiraceae*.

- 1) Family *Spirochaetaceae* includes two pathogenic genera to animals and human; genus *Treponema* and genus *Borrelia*.
- 2) Family *Leptospiraceae* includes one pathogenic genus to animals and human; genus *Leptospira*.

General characteristic of Spirochaetes:

- Spiral cells, long (5- 250 μm) and thin (around 0.1- 0.6 μm). Shape and number of the coils vary from genus to another
Coils of *Treponema*; 6-14 short regular coils with tapered ends.
Coils of *Borrelia*; 4-8 wide irregular coils.
Coils of *Leptospira*; Many fine tightly coils with hooks at one or both ends.
- Gram-negative bacteria but takes the stain poorly, only *Borrelia* can be easily stained by Gram stain, so special stains are used for their demonstration as silver impregnation, Fontana stain, and Giemsa stain.
- They are demonstrated effectively by using darkfield microscope and fluorescent microscope (?). *Borrelia* can be easily demonstrated by light microscope.
- They are motile by periplasmic flagella (**endoflagella**). Number of endoflagella differs according to genus and species. Also, motility differs from genus to genus
Motility of *Treponema*; rotating and undulating.
Motility of *Borrelia*; corkscrew like movement.
Motility of *Leptospira*; highly motile (Fast spiral rotation).
- **Air requirement**
Borrelia; microaerophilic.
Leptospira; Aerobic.
- **Cultivation on artificial media**
Treponema; can't grow on artificial media while can be propagated in embryonated eggs or laboratory animals.
Borrelia; grow on complex liquid or semi-solid media containing N-acetylglucosamine and long chain fatty acids, blood or tissue. Also, they can be propagated in embryonated eggs or laboratory animal.
Leptospira; grow on complex semi-solid media containing serum, vitamin B₁ and B₁₂. Also, they are propagated in embryonated eggs or laboratory animals.

Genus *Treponema*

Pathogenic *Treponema* species and diseases caused by them:-

- *Brachyspira paraluis-cuniculi* (*Treponema cuniculi*) causes **Rabbit syphilis, Vent disease, Rabbit spirochaetosis or Treponematosi**s in rabbits. Highly contagious disease characterized by presence of small nodules, ulcers or crusty scab- like lesions in the genital regions, nose, mouth, lips, chin and eyes. It is **venereal** and **zoonotic** disease.

- *Treponema pallidum* causes syphilis in human. It is **venereal** disease.

Laboratory diagnosis of Rabbit syphilis:-

- 1) **Sample:** Skin scraping or pus from the lesions.
- 2) **Direct detection and identification of *Treponema cuniculi* in the sample by**
 - **Dark field microscope** → thin long spiral cells with short regular coils and tapered ends showing rotating undulating motility.
 - **Light microscope** after staining with Fontana stain (or silver impregnation) → Bowen thin long spiral cells with short regular coils and tapered ends against yellowish background.
 - **Fluorescent microscope** using fluorescein labelled *T. cuniculi* Abs.
- 3) **Serologic tests for detection of *T. cuniculi* Abs.**
- 4) **PCR.**

Treatment of syphilis:-

Penicillin is the drug of choice.

Genus *Borrelia*

Many of *Borrelia* species are non-pathogenic and commensals but some of them cause systemic infections. They are transmitted to animals and human by arthropods (Tick and louse).

Borrelia species and diseases caused by them:-

- *Borrelia anserine* or *Borrelia gallinarum* causes **avian borreliosis**, **avian spirochaetosis** or **fowl spirochaetosis** in birds (chickens, turkey, pheasants, ducks, geese and wild birds). It is characterized by fever, emaciation, cyanosis of the head, greenish diarrhea and hepatosplenomegaly with widespread haemorrhages (**Mottled spleen**). *Borrelia anserine* is transmitted to the birds by soft ticks of genus *Argus* (important reservoir of infection), through **cannibalism parasitism** and ingestion.
- *Borrelia burgdorferi* causes **Lyme disease** or **borreliosis** in dogs mainly and other animals as cats, horses, cows & goats. It is characterized by fever, lameness and swollen lymph nodes.

Laboratory diagnosis of avian borreliosis and Lyme disease:-

- 1) **Sampling:** Blood or infected tissues (liver, spleen & L. nodes).
Borrelia anserine present in blood after 3-4 days of infection and disappear from blood before death.
- 2) **Microscopical examination:**
 - a) **For film stained with Giemsa, Gram or Fontana**
 - By Gram stain → Gram-negative, long thin spiral cells with wide irregular coils.
 - By Giemsa stain → pink, long thin spiral cells with wide irregular coils.

By Fontana stain → brown long thin spiral cells with wide irregular coils against yellowish background.

b) For smear treated with Fluorescein-labeled anti-*Borrelia* Abs by fluorescent microscope for direct detection of *Borrelia* cells.

3) Isolation and identification:

Cultivation in liquid or semisolid media containing N- acetylglucoseamine and long chain fatty acids, blood or tissue as Barbour- Stoenner-Kelly (BSK-II) and incubation under microaerophilic condition at 30-35 °C with monitoring growth of *Borrelia* by dark field microscopy for 4-6 weeks.

Or inoculation of the sample in Yolk sac of 5-6 days embryonated eggs and detection of *Borrelia* by dark field microscopy in the embryonic fluids few days post inoculation

4) Serologic test as ELISA for detection of Anti- *Borrelia* Abs.

5) PCR

Prevention and control of avian borreliosis and Lyme disease:-

1- **Treatment** of the infected cases by doxycycline and amoxicillin.

2- Vaccination:

For protection of birds from **avian borreliosis**, they are vaccinated by **Heat killed oil adjuvant vaccine or embryonated egg propagated *B. anserina* vaccine.**

For protection of dogs from **Lyme disease**, they are vaccinated by **whole-cell killed vaccine** after 12 weeks of age and it is recommended to give two doses three weeks apart then given boosted dose yearly.

3- Tick eradication is the most important measure in prevention and control of avian borreliosis and Lyme disease.

Genus *Leptospira*

Leptospira species are widely distributed in the nature particularly where the water found, so stagnant or slow moving water are predisposing factors for Leptospirosis.

Classification of *Leptospira*:

Through DNA homology, *Leptospira* is classified into two species; *L. interrogans* and *L. biflexa*.

1) *L. interrogans* contains the pathogenic *Leptospirae* and on basis of serological reactions, currently there are 250 serovars within 23 serogroups.

2) *L. biflexa* contains the saprophytic *Leptospirae*.

Common *Leptospira* serovars and diseases caused by them:

► *L. interrogans* serovar *interrogans* causes abortion and mastitis in cattle and sheep.

- ▶ *L. interrogans serovar pomona* causes Red water fever in calves & lambs and abortion in cattle, sheep, horses and pigs.
- ▶ *L. interrogans serovar icterohaemorrhagiae* causes fever, haemoglobinuria and abortion in cattle, sheep and goat.
- ▶ *L. interrogans serovar canicola* causes acute hepatitis with jaundice in dog (**Canicola fever in dogs**) and human (**Weil's disease**).
- ▶ Many other serovars of *Leptospira* causes abortion and stillbirths in the domestic animals.

Laboratory diagnosis of Leptospirosis:

- 1) **Sampling;** urine, blood, aborted feti, fetal fluids, fetal membranes, and milk.
- 2) **Detection of *Leptospira* by dark field microscopy or FAT** in the blood (during the early days of infection; Leptospiremia), urine (later; Leptosuria, for 2-3 months) or tissues --- Thin long tightly coiled spiral cells with hooks at one or both ends.
- 2) **Isolation and identification**
Cultivation in semisolid media containing serum, vitamin B₁ and B₁₂ and incubation under aerobic condition or inoculation of the sample into embryonated eggs and detection of *leptospira* by dark field microscopy.
- 3) **Serological test**
 - **ELISA** to detect anti-*leptospira* antibodies in serum.
 - **Microscopic agglutination test;** the most common test, serum dilutions are titrated. Titer above 1:400 or for four-fold rise in the titer in paired samples is indicative for the infection.
- 4) **PCR.**

Treatment of Leptospirosis:

Penicillin will quickly stop leptospira from replicating but will not clear the carrier state.

Obligatory intracellular scotobacteria

Chlamydiae and **Rickettsiae** are gram negative bacteria but they are **obligate intracellular parasites** (resemble viruses in this character) therefore, they are grown in tissue culture, embryonated eggs or laboratory animals. The obligate intracellular parasitism of **Chlamydiae** is due to that they can't synthesize ATP while that of **Rickettsiae** is due to that they are unable to produce sufficient ATP to replicate extracellularly and they require the rich cytoplasm to stabilize their unusually permeable cell membrane.

I- Order Chlamydiales

Life cycle of Chlamydia:

They multiply by a special developmental cycle include binary fission as follow

- 1) **The elementary body** (Infectious form) is taken into the cell by phagocytosis-like process.
- 2) Inside the cell, the **elementary body** form larger **initial or reticulate body**.
- 3) The **initial or reticulate body** grows in size and divides by binary fission forming a large number of **elementary bodies** (these are seen as intracytoplasmic inclusion bodies in host cells).
- 4) On lysis of the host cells, **the elementary bodies** are released to infect new cells.

Morphology and culture characteristics:

- Gram negative bacteria but stained better with Giemsa or Macchiavello stain.
- Spherical small cells (0.5um).
- They are grown in tissue culture as HEP-2 cells, embryonated eggs (Yolk sacs of 6-8 day old) or laboratory animals as mice. This where they are obligate intracellular parasites.

Chlamydia species and their infections:

- ***Chlamydophila psittaci*** (formerly *Chlamydia psittaci*) causes **Parrot disease, Parrot fever, Ornithosis, Avian chlamydiosis or Psittacosis** in parrots, sparrows, gulls, pigeons, ducks, and hens. This disease is characterized by affection of upper respiratory tract and yellowish green or grayish watery diarrhea with high level of urates. In human, and *Chlamydophila psittaci* causes atypical pneumonia (Zoonotic disease).
- ***Chlamydophila psittaci*** is one of the major causes of **abortion** in sheep and cattle.
- ***Chlamydia pneumoniae*** causes **atypical pneumonia** in human.
- ***Chlamydia trachomatis*** causes **ocular, genital and respiratory infections** in human.

Laboratory diagnosis of Parrot disease:

1) Sampling:

Nasal discharges, faeces, blood, smears from liver, cloaca & spleen of dead birds.

- 2) **Microscopical examination for film from samples stained with Giemse or Macchiavello stains** → Intracytoplasmic inclusions bodies "elementary and reticulate bodies".

Or detection of these Intracytoplasmic inclusions bodies by IFT.

- 3) **Isolation of *C. psittaci* on tissue culture as HEP-2, egg yolk of 6-8 day old chicken embryo or laboratory animal as mice** → Intracytoplasmic inclusion bodies in the cells.
- 4) **Detection of Chlamydial Abs by CFT test or ELISA.**
- 5) **PCR.**

Treatment of chlamydial infections:

Usually by **doxycycline** or **tetracycline**.

II- Order Rickettsiales

Rickettsiae are maintained in the nature in certain arthropods as ticks, lice, and fleas and they are transmitted to human and animals by bite or faeces of the arthropod (**arthropod borne infection**) except Q fever which is transmitted by inhalation or ingestion.

Morphology and culture characteristics:

- They are short Gram negative bacilli, difficult to be stained by Gram but are best stained by Giemsa, Leishman or Macchiavello stain.
- They are grown in tissue culture, embryonated eggs or laboratory animals. This where they are obligate intracellular parasites.

Rickettsiae and their infections:

- ***Rickettsia rickettsii* (*R. rickettsii*)** causes **Rocky Mountain spotted fever** in dogs and rodents. This disease is characterized by fever and appearance of petechiae and edema in the mucosal surfaces, extremities, scrotum and the ears. It is transmitted by ticks. ***R. rickettsii*** accidentally affects human (Zoonotic disease).
- ***Coxiella burnetii* (*C. burnetii*)** causes **Q fever, Query fever, Queensland rickettsial fever or Abattoir fever** in cattle, sheep and goats. This disease is characterized by placentitis and abortion. ***C. burnetii*** affects human (**Zoonotic occupational disease**) causing influenza like symptoms and hepatitis.
- ***Anaplasma marginale*** causes **Anaplasmosis** in cattle and buffaloes.
- ***Rickettsia prowazekii*** causes Epidemic typhus in human.
- ***Rickettsia typhi*** causes Endemic typhus in human.

Laboratory diagnosis of Rickettsial diseases:

- 1) **Sampling:** according to the disease.
- 2) **Microscopical examination for film from samples stained with Giemse or Macchiavello stains** → Rickettsial bodies.
Or detection of Rickettsial bodies by IFT.
- 3) **Serological test as CFT test or ELISA to detect the antibodies against the causative agent.**
- 4) **PCR.**

Treatment of Rickettsial diseases:

Antibiotics used for treatment of Rickettsial diseases includes are doxycycline, tetracycline, chloramphenicol and ciprofloxacin.