

**2<sup>nd</sup> Professional Year, Veterinary Microbiology (Unit-I)**

# **YERSINIA, PROTEUS**

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**DR. SAVITA KUMARI**  
**ASSISTANT PROFESSOR-CUM-JR. SCIENTIST**  
**DEPARTMENT OF VETERINARY MICROBIOLOGY**  
**BIHAR VETERINARY COLLEGE, BASU, PATNA**

# YERSINIA SPECIES

- Rod shaped, facultative anaerobic Gram-negative bacteria, non-lactose fermenters
- Motile (exception *Y. Pestis*)
- *Y. pestis*, *Y. enterocolitica* and *Y. pseudotuberculosis* are pathogenic for animals and humans
- *Y. ruckeri* - pathogen of fish
- Growth of yersiniae tends to be less rapid than other members of the *Enterobacteriaceae*
- Characteristically demonstrate bipolar staining in Giemsa-stained smears from animal tissues
- *Yersinia* species on blood agar- Greyish, round and shiny, 2.0–3.0 mm, nonhaemolytic colonies

- Serotyping and biotyping methods are used for identifying pathogenic yersiniae
- 21 serological groups of *Y. pseudotuberculosis*, serotypes I, II and III majority of pathogenic isolates
- Five biotypes and more than 50 serotypes of *Y. enterocolitica*
- Somatic antigens 2, 3, 5, 8 and 9 in isolates from clinical infections caused by this species
- Serotype O:9- important, shares common antigens with *Brucella* species and may induce false-positive reactions in brucella agglutination tests

# DISEASES:

<i>Yersinia</i> species	Hosts	Consequences of infection
<i>Y. enterocolitica</i>	Pigs, other domestic animals, wildlife	Subclinical enteric infections, occasionally enteritis
	Ewes	Sporadic abortion
	Humans	Gastroenterocolitis
<i>Y. pseudotuberculosis</i>	Farmed deer, sheep, goats, cattle, buffaloes, pigs	Enteritis in young animals, subclinical infections common in older animals, mesenteric lymphadenitis
	Cattle, sheep, goats	Sporadic abortion
	Guinea-pigs, other laboratory animals	Focal hepatic necrosis, septicaemia
	Caged birds	Septicaemia
	Humans	Enterocolitis, mesenteric lymphadenitis
<i>Y. pestis</i>	Humans	Bubonic and pneumonic plague
	Rodents	Sylvatic plague
	Cats	Feline plague

(Quinn et al.,2011)

## *YERSINIA PSEUDOTUBERCULOSIS* AND *Y. ENTEROCOLITICA*

- Found in the intestinal tract of a wide range of wild mammals, birds and domestic animals
- May be reservoirs of infection
- Many avian species may act as amplifier hosts and may also transfer organisms mechanically
- Both organisms can grow in a wide temperature range (5 to 42°C)
- Survive for long periods in cool wet conditions

## ***Y. pestis***

- In endemic areas, wild rodents important reservoirs
- Fleas, especially *Xenopsylla cheopis*, the Oriental rat flea, transmit the infection to humans and other animals
- Following ingestion by the flea from a bacteraemic host, the organism multiplies within the gastrointestinal tract of the flea resulting in blockage of the tract
- Effectively deprives flea of nutrients, which drives it to continually seek other animals on which to feed
- During feeding the flea regurgitates yersiniae into the bite wounds on these new hosts

- *Yersinia pestis* maintained by a sylvatic cycle in wild rodents and their associated fleas
- Dog and cat fleas may become infected with *Y. pestis*
- Human infections through cuts, bites, scratches and aerosols can occur
- Cats susceptible to *Y. pestis* and naturally infected cats can pose a health hazard for humans in endemic areas
- Pigs- carriers of *Y. enterocolitica* strains that are pathogenic for humans

# PATHOGENESIS

- Fleas feeding on bacteraemic hosts ingest *Y. pestis*, which multiplies and blocks the proventriculus of the flea
- This in turn leads to regurgitation of the organism when the blocked flea repeatedly attempts to feed on other animals which then become infected
- In addition, infection may occur through contact with infected secretions or tissues, for example, cats may become infected by oral exposure through ingestion of infected rodents
- In humans, infection may occur by exposure to infected aerosols from other cases of pneumonic plague either in humans or cats
- Infection of enteropathogens *Y. pseudotuberculosis* and *Y. enterocolitica* - by ingestion



# VIRULENCE FACTORS

- 70-kb virulence plasmid which encodes a type III protein secretion system and *Yersinia* outer proteins or Yops
- Type III secretion system exports the Yops from the cell, several of which are delivered directly into phagocytes where they inhibit phagocytosis and proinflammatory cytokine production
- More pathogenic strains of yersiniae encodes yersiniabactin, a siderophore which provides the organism with iron and important for multiplication within host
- Pathogenic yersiniae - facultative intracellular organisms and survival within macrophages important particularly in the early stages of infection

- Two plasmids - specific to *Y. pestis*, important for virulence
- One plasmid - production of capsule increases resistance to phagocytosis
- also contains a gene for production of phospholipase D, important for multiplication in midgut of flea
- Other plasmid - plasminogen activator, helps dissemination of organism from flea bite area
- *Y. pestis* produces extra virulence factors, more pathogenic than *Yersinia pseudotuberculosis* and *Y. enterocolitica*

- Chromosomally encoded invasion gene (*inv*) in enteropathogenic yersiniae, assists in translocation of the bacteria across the intestinal wall and colonization of Peyer's patches
- Once in the mucosa, the bacteria are engulfed by macrophages in which they survive and are transported to the mesenteric lymph nodes
- Replication in the lymph nodes follows with development of necrotic lesions and neutrophil infiltration
- Endotoxin also contributes to the pathogenesis of disease

# CLINICAL INFECTIONS

## *Yersinia enterocolitica*

- Wild and domestic animals may act as reservoirs, Primarily a human enteric pathogen
- Pig natural reservoir for *Y. enterocolitica* serotype O3 biotype 4, important pathogen in humans
- Rare cases of enteric disease, precipitated by stress, may be encountered in pigs, farmed deer, goats and lambs
- Sporadic ovine abortion

## *Yersinia pestis*

- Cause of human bubonic plague ('black death')
- Can infect both dogs and cats in endemic areas
- Cats, particularly susceptible, may be a source of infection for owners and attending veterinarians

## *Yersinia pseudotuberculosis*

- Enteric infections in a wide variety of wild and domestic animals, often subclinical
- Septicaemic form of disease (pseudotuberculosis) can occur in laboratory rodents and aviary birds
- Sporadic abortion in cattle and goats

# ENTERIC YERSINIOSIS

- Enteritis caused by *Y. pseudotuberculosis*
- Farmed deer, in buffaloes, sheep, goats and cattle
- Subclinical infection in many species common
- Clinical disease may be precipitated in the winter months by stress factors such as poor nutrition, weaning, transportation and cold wet conditions
- There may be prolonged survival of *Y. pseudotuberculosis* on pasture in cold wet weather, facilitating faecal–oral transmission

- Characterized by profuse watery diarrhoea, sometimes bloodstained, may be rapidly fatal if untreated
- The luminal contents of the small and large intestine watery, and mucosal hyperaemia evident at post-mortem examination
- Severely affected animals may show mucosal ulceration
- Mesenteric lymph nodes often enlarged and oedematous
- Scattered pale necrotic foci may be present in the liver
- A clinically similar but less severe enterocolitis caused by *Y. enterocolitica* in young ruminants

# DIAGNOSIS

- The species and age group affected, especially during cold wet spells of weather, may suggest yersiniosis
- Histological examination of intestinal lesions may reveal clusters of organisms in microabscesses within the mucosa
- Confirmation requires isolation and identification of *Y. pseudotuberculosis* or, occasionally, *Y. enterocolitica*:
- Samples from tissues can be plated directly on to blood agar and MacConkey agar and incubated aerobically at 37°C for up to 72 hours
- Faecal samples should be plated directly on to special selective media containing antibiotics which suppress the growth of contaminating organisms
- A cold enrichment procedure may facilitate recovery of yersiniae from faeces, especially if they are present in low numbers
- A 5% suspension of faeces in phosphate buffered saline, held at 4°C for 3 weeks, sub-cultured weekly on to MacConkey agar or selective agar
- PCR-based identification techniques



# TREATMENT AND CONTROL

- Fluid replacement therapy together with broad spectrum antimicrobial treatment
- A formalin-killed *Y. pseudotuberculosis* vaccine composed of serotypes I, II and III, administered in two doses 3 weeks apart, has been shown to decrease the occurrence of clinical disease in young deer
- Recent developments include the successful use of a recombinant intranasal vaccine which provides mucosal and systemic protection against *Y. pseudotuberculosis* in mice
- Stressful conditions should, where practicable, be minimized

## **SEPTICAEMIC YERSINIOSIS**

- Septicaemia, caused by *Y. pseudotuberculosis*
- Occurs in birds kept in cages or aviaries
- Infection is acquired through contact with the faeces of wild birds or rodents, or through the feeding of contaminated leafy plants
- In aviaries, overcrowding may predispose to the development of disease
- Infected birds may die suddenly
- Some may display ruffling of feathers and listlessness shortly before death
- Pin-point white necrotic foci - liver at post-mortem examination
- Confirmation - isolation and identification of *Y. pseudotuberculosis* from liver, other internal organs
- Treatment- seldom feasible
- Control – prevention of faecal contamination of food and water by wild birds and rodents

# **PSEUDOTUBERCULOSIS IN LABORATORY ANIMALS**

- Infection with *Y. pseudotuberculosis* in colonies of guinea-pigs or rodents
- Through faecal contamination of food by wild rodents
- Diarrhoea and gradual weight loss leading to emaciation and death
- Some animals may die suddenly from septicaemia
- At PM- numerous white necrotic lesions in liver, affected mesenteric lymph nodes enlarged and may show caseous necrosis
- Treatment- usually undesirable, some animals in colony may become carriers, zoonotic
- Depopulation, disinfection and restocking - preferred control measures
- Exclusion of wild rodents

# ***FELINE PLAGUE***

- Cats usually acquire infection with *Y. pestis* by ingestion of infected rodents
- Three clinical forms: bubonic, septicaemic and pneumonic
- **Bubonic form-**
- Most common
- Characterized by enlarged lymph nodes (buboes) associated with lymphatic drainage from the site of Infection
- Clinical signs include fever, depression and anorexia
- Affected superficial lymph nodes may rupture, discharging sero-sanguineous fluid or pus

- **Septicaemia-** may occur without lymphadenopathy and potentially fatal

### **Pneumonic lesions-**

- May result from haematogenous spread
- Cats with pneumonic lesions potential source of human infection through aerosol generation, they should be euthanized
- Human infection can also be acquired through cat scratches and bites and possibly through the bites of fleas from infected cats
- Care should be taken when handling infected animals

# DIAGNOSIS

- Lymphadenopathy and severe depression in cats in endemic areas may suggest feline plague
- Suitable specimens - pus, blood and lymph node aspirates
- Giemsa-stained smears from abscesses or lymph node aspirates may reveal large numbers of bipolar staining rods
- Direct fluorescent antibody tests
- PCR-based techniques
- A passive haemagglutination test, using Fraction IA antigen, can be used on paired serum samples taken 2 weeks apart from suspect cats, substantial increase in antibody level usually indicative of active infection

# TREATMENT AND CONTROL

- Cats with suspected plague should be kept in isolation
- immediately treated for fleas to prevent those handling the animal from becoming exposed to flea bites
- The bubonic form of the disease may respond to tetracyclines or chloramphenicol administered parenterally
- In endemic areas, dogs and cats should be routinely treated for fleas
- Rodent control measures should be implemented after flea control procedures are in place

	<i>Yersinia</i> species	<i>Proteus</i> species
Clinical importance	Major pathogens	Opportunistic pathogens
Cultural characteristics	–	Swarming growth <sup>a</sup>
Motility at 30°C	Motile <sup>b</sup>	Motile
Lactose fermentation	–	–
IMViC tests		
Indole production	v	± <sup>c</sup>
Methyl red test	+	+
Voges-Proskauer	–	v
Citrate utilization test	–	v
H <sub>2</sub> S production in TSI agar	–	+
Lysine decarboxylase	–	–
Urease activity	+ <sup>b</sup>	+

a, when cultured on non-inhibitory medium.

b, except *Y. pestis*.

c, *P. vulgaris* +; *P. mirabilis* –.

v, reaction varies with individual species

(Quinn et al.,2011)



## triple sugar iron (TSI) agar

Species	pH change <sup>b</sup>		H <sub>2</sub> S production
	Slant	Butt	
<i>Salmonella</i> serotypes <sup>c</sup>	Red	Yellow	+ <sup>d</sup>
<i>Proteus mirabilis</i>	Red	Yellow	+
<i>P. vulgaris</i>	Yellow	Yellow	+
<i>Escherichia coli</i>	Yellow	Yellow	-
<i>Yersinia enterocolitica</i>	Yellow	Yellow	-
<i>Y. pseudotuberculosis</i> and <i>Y. pestis</i>	Red	Yellow	-
<i>Enterobacter aerogenes</i>	Yellow	Yellow	-
<i>Klebsiella pneumoniae</i>	Yellow	Yellow	-

b, red, alkaline; yellow, acid.

c, *Salmonella* serotypes and *Proteus* species can be differentiated by lysine decarboxylase production and urease activity

(Quinn et al.,2011)

# PROTEUS SP.

- Gram-negative bacteria, members of *Enterobacteriaceae* family
- Opportunistic pathogens, cause infections of the lower urinary tract in dogs and horses
- Often implicated in otitis externa in dogs and sometimes in cats
- On Blood agar- grey, swarming growth over the agar, swarming can be in waves, turns blood agar brown, very foul smell colonies
- Most *Proteus mirabilis* and *P. vulgaris* strains will swarm on blood agar
- Pale and discreet on MacConkey agar but edges may be irregular
- Most *P. vulgaris* and *P. mirabilis* strains produce hydrogen sulphide in TSI and XLD media

## **DIAGNOSTIC PROCEDURES**

- Specimens for examination should be collected from the infected organ
- Blood agar and MacConkey agar
- Identification criteria for isolates:
  - Gram-negative rods
  - Oxidase-negative, catalase positive, nitrate positive, urease positive
  - Growth and appearance on MacConkey agar
  - Colonial appearance on blood agar.
  - Appropriate biochemical profile for presumptive or definitive identification

## ***Treatment and control***

- The type of treatment is determined by the location and severity of the infection
- Antibiotic therapy should be based on antibiotic susceptibility testing
- Predisposing causes and sources of infection should be identified and, if possible, eliminated