Bacteriology, Immunology and Mycology department

Third year (general program)

2020

Family: PASTEURELLACEAE

Members of the family Pasteurellaceae are:

- Gram negative short rods, non sporulated, facultative anaerobes with fermentative metabolism.
- Some of them are parasitic to the mucous membranes of mammals and birds.

The family Pasteurellaceae is differentiated into genera:

1. Genus *Pasteurella:*

- It characterized by the bipolarity in films from infected tissues, parasitic in respiratory tract.

2. Genus *Haemophilus:*

- It requires for its growth two factors (V and X). The V-factor is present in haematin i.e. chocalate or boiled blood, while the X – factor occurs in fresh substances and liquid (vitamin C in structure). Strict parasitic.

3. Genus *Actinobacillus:*

- It does not need V or X - factors. Actinobacillus grow on nutrient agar with glucose.

The differential characters of related genera with *pasteurella*:

Characters	Pasteurella	Yersenia	Heamophilus	Actinobacillus
Bipolarity	+	–	–	–
Requirement of X and V factors	–	–	+	–
Sticky colonies	–	–	–	+
Growth on Macconkey agar	–	+	–	+
Growth on 4.5% Nacl	–	+	–	–
Catalase test	+	+	–	+/-
Oxidase test	+	+	–	–
Nitrate reduction	–	+	–	–

Family: PASTEURELLACEA

Genus: PASTEURELLA

Species: *Pasteurella multocida*

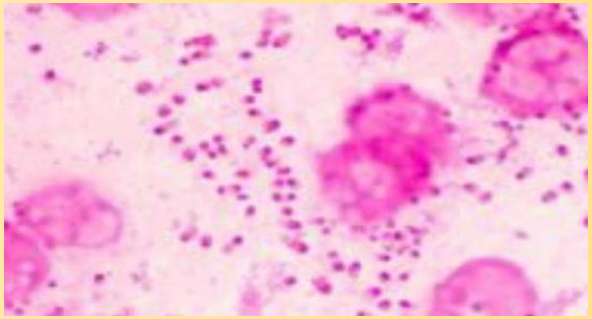
Pasteurella are commensally in the upper respiratory tracts of clinically normal animals. They are parasitic on the mucous membranes of respiratory and digestive system of mammals (rarely man) and birds.

Pasteurella multocida causes some serious outbreaks in different types of animals as primary infection as:

1. Haemarrhagic septicaemia in bovine
2. Fowl cholera acute or chronic forms in fowls
3. Pasteurellosis in different animals especially in young animals.
4. Also it may play a role as a secondary invaders of pneumonia in different species of animals.

Morphology and staining characters:

- Gram negative, ovoid to short rods, coccobacilli (0.3-1 μ x 1.3 μ)
- Pasteurella arranged singly or short chains.
- Pasteurella in blood or in tissue films stained by leishman's stain give the characteristic bipolarity character due to presence of Volutin (metachromatic) granules.



- Non-motile, non sporulated.
- Most of the virulent strains of *pasteurella multocida* have slime layer composed of complex polysaccharide, it is mucoid or smooth.

Cultural characters:

- *Pasteurella* is facultative anaerobes.
- They grow well on the presence of blood or serum at 37°C after 24 hours incubation.
- **In serum broth, glucose broth:** slight turbidity after (24-48) hours with sediment.

➢ **On sheep blood agar:** the growth of *Pasteurella multocida* from fresh materials develops after (24-36) hours in three forms:

1- Mucoid colonies:

They are round, convex, sticky large 2-3mm. in diameter, mucoid in consistency with large amount of slime layer of hyaluronic acid. Highly virulent Strains for rabbits and pigeons

2- Smooth (iridescent colonies)

1 mm, in diameter, round, grayish, glistening, possesses small quantities of slime layer; the strains are of moderate virulence for mice and rabbit.

3- Rough or blue variant:

Small in consistency, isolated mainly from chronic cases or after repeated subcultures. The rough colonies show auto-agglutination due to the loss of the slime layer Also it may be a virulent to rabbit

Mucoid, non-haemolytic colonies of *P. multocida* on blood agar

Biochemical characters:

- It is catalase +ve ,oxidase + ve.
- It is essential to differentiate between Pasteurella multocida and other members of pasteurella speciesBiochemical differentiation between species of pasteurella:

Species	Lactose	Mannitol	B-haemolysis	Growth on Macconkey	Indol	H2S	Urea
P. multocida	-	+	-	-	+	-	-
P. haemolytica	+	+	+	+	-	-	-
P. gallinarum	-	-	-	-	-	-	-
P. aerogenes	-	-	-	+	-	-	+

Biochemical characters:

Pasteurella multocida possesses 2 types of bacterial antigens

- Somatic (O)-antigen which differentiated to 1-1 1 types.
- Capsular (K) antigens (surface antigens)
- The K-antigen differentiated by Carter to5 types (A, B, C, D and E).

It is clear that *Pasteurella multocida* consists of more than 16 serotypes some of them are associated with certain animal species. The serotype is designated by its capsular type (A, B, ,D and E) and by number indicating the somatic type as B:2, E:2, A:8, A:5 as indicated in the following table:

Serological classification of *Pasteurella multocida* and susceptible animals to each serotype:

Roberts 1947	**I**	**II**	**III**	**IV**	
Carter 1955	**A**	**B**	**C**	**D**	**E**
Susceptible animals					
	Fowl Cattle Pig	**Cattle**	**Un-known**	**Sheep Pig**	**Cattle**
Serotypes					
	A:3 A:5 A:8 A:9	**B:6**		**D:1 D:4**	**E:6**

Diseases caused by *Pasteurella multocida*:

- **Cattle and buffaloes:** Haemorrhagic septicemia
- **Sheep:** Pleuropneumonia - Mastitis
- **Horse:** Contagious pleuro-pneumonia.
- **Rabbits:** Snuffle (pneumonia) - Chronic abscess
- **Fowls:** Fowl cholera

Laboratory diagnosis:

1- History, symptoms and P.M lesions.

It is usually confused especially with septicemic diseases of large animals or sheep. Bacteriological examination is essential to isolate the causative organism.

❖ Samples required:

- In acute septicemic cases, all parenchymatous organs are severely congested and harbored the organism in pure form as liver, heart blood, spleen, kidney and lungs and make films.
- In purified carcasses, pasteurella multocida present in pure form in bone marrow.
- In chronic cases, the organism present in the affected lesion in association of other bacteria especially in case of wattle disease in poultry.

2- Isolation and identification of *P. multocida* :

Isolation of *Pasteurella multocida* in pure form on blood agar, selective media as DAS and Morres media. Followed by biochemical identification.

(Morphological, cultural, biochemical characters previously discussed)

3- Staining of tissue films with Leishmans stain:

Films from blood or tissues showed characteristic bipolarity of Pasteurella multocida due to presence of metachromatic granules.

4- Demonstration of the pathogenicity of the isolates.

It is very essential to determine the virulence of the isolates to mice; also it is diagnostic to demonstrate the bipolarity in its blood.

5- Molecular identification.

Differential diagnosis:

In case of fowl cholera, it must be necessary to apply differential laboratory diagnosis between the following diseases of poultry causing high mortality in poultry species.

- New castle disease.
- Fowl influenza
- Fowl typhoid or pullorum disease.

Immunization and control:

➢ Active immunization

It is very practical using:

- Formalized whole culture vaccine.
- It is prepared from the isolated smooth colonies of the serotype associated.
- Alum precipitated vaccine:
- It may contain other adjuvant to give immunity for one year.

➢ Passive immunization:

It is of no value due to rapid course of the disease.

Haemophilus

Haemophilus species are assigned to the family *pasteurellaceae* and are common commensally organisms of mucous membrane of animal and human.

- Member of this genus requires certain growth factors present in blood (Haemophilic) for their growth.
- The most important species for human and domestic animals are:

1- *H. influenzae*: human.

2-*H. agni*: avium.

3- *H. parasuis:* pigs.

4- *H. paragallinarum*: chicken.

5- *H. somnus*: bovine.

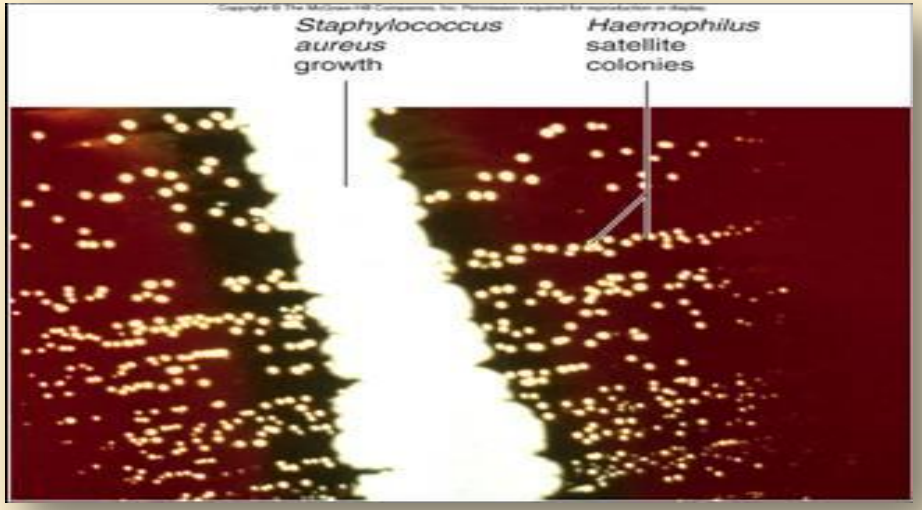
Morphology:

Gram negative, small coccobacilli, non motile, non capsulated, non sporulated.

Culture characteristics:

- The pathogenic Haemophilus species of domestic animals are facultative anaerobe at 37° C.
- Most Haemophilus require factor X and / V factor for in vitro growth.
- X factor (haemin) and V factor (NAD) nicotinamide adenine dinucleotide.
- Some species of Haemophilus require both X and V factors, only X or V factors, or neither.
- The Haemophilus species not grow on ordinary media.
- It grow on blood agar and chocolate agar which provide its growth factors, colony is very small droplet like.
- Some species are haemolytic and other non haemolytic.

- **On blood agar:** smooth colonies, small, translucent, non haemolytic. And with Staph. aureus streak lines, it makes satellitism phenomena.



- **On selective media (chocolate agar or heated blood agar):** Haemophilus species need one or both of two growth factors.

1- Heat stable X factor present in haemin of blood.

2- Heat labile V factor (NAD) nicotinamide adenine dinucleotide.

Biochemical characters:

Test	Result
Oxidase	-
Catalase	-
Sugar fermentation	Ferment galactose, manitol, glucose with acid production only.
MR	-
VP	-
Citrate	-
Urea hydrolysis	-
H2S	-
Oxidase reaction varies with species	- in H. parasuis
	+ in H. agni
	+ H. somnus

Pathogenicity:

- ***H. agni*** **:** cause ovine septicemia, polyarthritis, pneumonia and meningitis in sheep and goat.
- ***H. parasuis*** : cause polyserositis (glasser's disease) in pig.
- ***H. somnus*** **:** cause bovine thromboembolic meningeoencephalitis.
- ***H. mflueunzae*** **:** cause otitis media, sinusitis or pneumonia and meningitis in human.
- ***H. paragallinerum*** **:** cause infectious coryza in chicken.

Laboratory diagnosis:

1) Laboratory diagnosis depend on isolation of the organisms on heated blood agar.
2) Microscopic examination.
3) By satellite phenomenon of blood agar treated with *Staphylococcus aureus* provide both X and V factor.
4) Serological diagnosis by fluorescent antibody technique of the organism and counter immune-electrophoresis.

Thank you