Pasteurella & Manheimia

General features

- Pasteurellae are small (0.2 micron by upto 2 micron)
- Gram-negative rods or coccobacilli, show bipolar staining
- Non motile, non sporing.
- Facultative anaerobic, fermentative.
- Oxidase positive and catalsae positive.
- Best growth on media supplemented with serum or blood.

Natural habitat

- Worldwide distribution
- Wide spectrum of hosts
- Most commensals on mucous membranes of Upper respiratory tract and intestinal tracts of animals
- Buffaloes are generally more susceptible to HS than cattle and show more severe forms of disease with profound clinical signs.
- Subcutaneous oedema from the mandible to the brisket is one distinctive feature of the disease in endemic areas most deaths are confined to older calves and young adults.

Pathogenesis

- Pasteurella are extracellular pathogens.
- Stress like concurrent viral infection,
- Endotoxins important in septicaemic disease like fowl cholera HS.
- Infectious can be endogenous or exogenous.
- Portal of entry usually via reppiratory tract.
- Virulence enhanced in animal to animal transmission

Serogroups or types of *Pasteurella spp*.

- Basis for types or serogroups of *Pasteurella multocida* – **Capsular** substance.
- Carters group
- **A, B, D, E and F**

- **Type A** - Causes fowl cholera, pneumonia & other infections
- **Type B** - Causes Epizootic Haemorhagic septicemia in Asia, middle east & southern Europe
- **Type D** - Pneumonia and Atropic Rhinitis in swine
- **Type E** - Causes Haemorhagic Septicaemia in Africa
- **Type F** - Recovered from turkeys

- Somatic types have also been determined and given numbers
- Serotype identified by serogroup followed by its somatic type
- E.g bovine haemorhasgic septicaemia is B:6 in south east Asia and E:6 in africa.(Namioka-Carter system),
- Biotypes have differences in number of characteristics including pathogenicity, antigenic nature and biochemical activity
- E.g biotype A ferments arabinose and biotype t ferments trehalose

Laboratory diagnosis

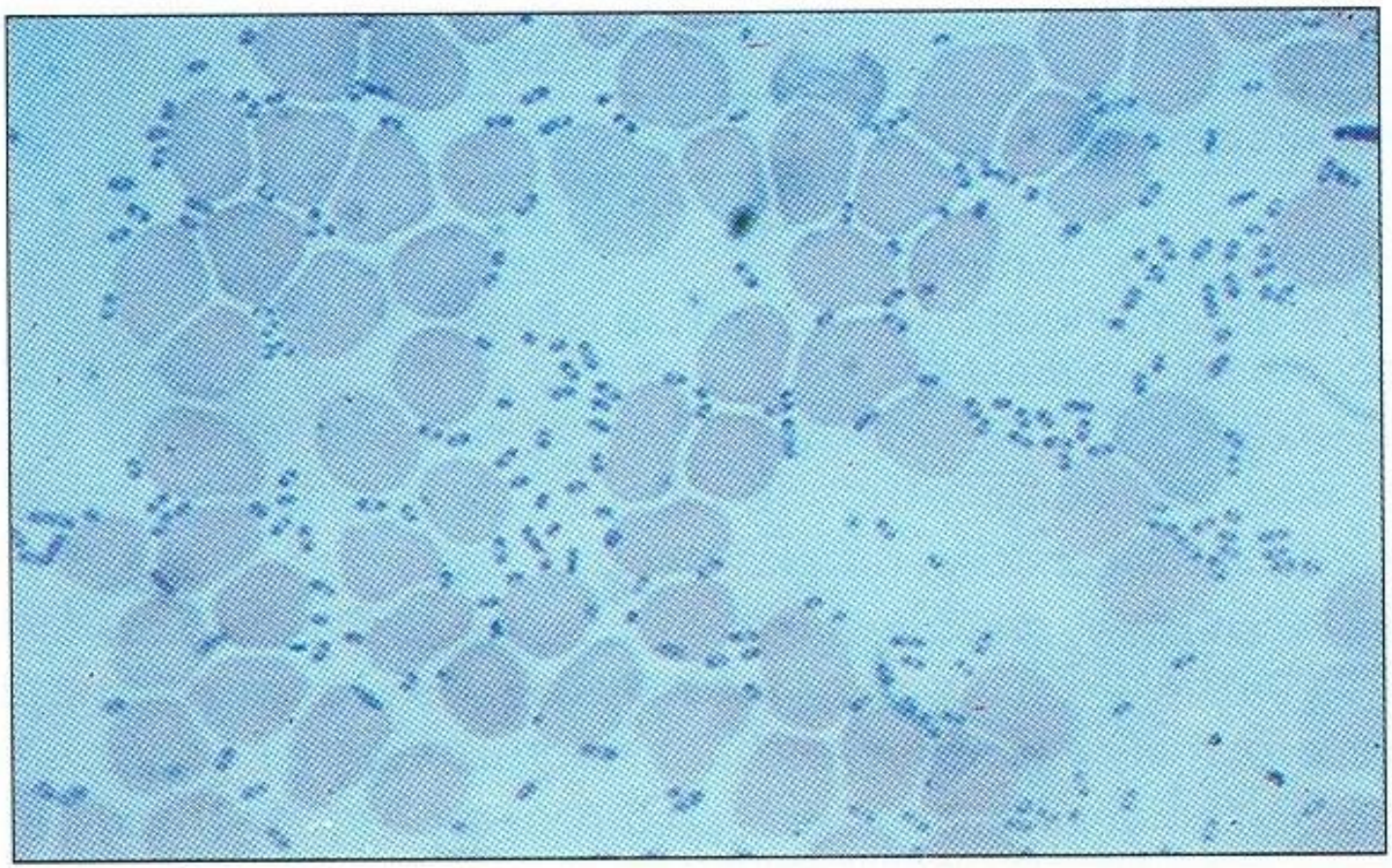
- **Specimens-**
- Depend on animal species and on disease condition
- Portion of pneumonic lung
- In case of septicaemia-pieces of liver, spleen, kidney and lymph node
- From live animals-pus, exudate, nasal swab, bronchial lavage and mastitic milk

Direct microscopy

- Small, Gram negative rods or coccobacilli

- In septicaemias (fowl cholera), distinctive bipolar-staining can be seen in Giemsa-satined or Leishmanstained smear

BIPOLAR STAINING OF *Pasteurella spp*.



318 *Pasteurella multocida* in a bovine blood smear from a case of haemorrhagic septicaemia showing the characteristic bipolar staining. (Leishman stain, ×1000)

Isolation

- Routine medium for isolation is Ox or Sheep blood agar.
- Clinical materials –inoculated on both blood and MacConkey agar
- Selective medium contain Clindamycin (2 microgram/ml) used for isolation from porcine nasal swabs
- Incubate aerobically at 37C for 24-48 hrs

Animal inoculation test

- Intraperitoneal inoculation of mice-necessary to recover P. Multocida from clinical specimens that contain large numbers of other bacteria

PRINCIPLE HOSTS AND DISEASE CAUSED BY *PASTEURELLA spp.*

Species	*Principal host(s)*	*Disease and commensal status*
P. multocida		
type A	Cattle	Part of 'shipping fever' complex Part of the 'enzootic pneumonia' complex in calves Occasional but severe mastitis
	Sheep	Pleuropneumonia Mastitis
	Pigs	Pneumonia (often secondary)
	Rabbits	One cause of 'snuffles', pleuropneumonia, abscesses, otitis media, conjunctivitis and genital infections
	Poultry	Fowl cholera (primary infection)
	Many domestic and wild animals	Pneumonia and other infections in stressed animals Commensals in respiratory and digestive tract
type B	Cattle, water buffalo, bison, yak and other ruminants	Epizootic haemorrhagic septicaemia (primary infection) Nasopharynx of carrier animals South-East Asia and other countries
type D	Pigs	Atrophic rhinitis (with or without *Bordetella bronchiseptica*)
	Pigs, less commonly other domestic animals and poultry	Pneumonia (usually secondary)
type E	Cattle and water buffalo	Epizootic haemorrhagic septicaemia (primary infection) Africa only
type F	Turkeys mainly	Role in disease not clear

P. haemolytica *biotype A*	Cattle	Part of 'shipping fever' complex Pneumonia (primary or secondary)
	Sheep	Enzootic pneumonia (primary or secondary). Septicaemia in lambs under 3-months-old. Gangrenous mastitis ('blue bag')
biotype T	Sheep	Septicaemia in lambs 5–12 months-old
P. pneumotropica	Rodents	Pneumonia (secondary) and abscesses (possibly from bites) Commensal in nasopharynx
	Dogs and cats	Present in the nasopharynx of some animals. Not a significant pathogen
P. canis	Dogs (man)	Recovered from the oral cavity of dogs and from dog bites in humans
P. dagmatis	Dogs, cats (man)	Present in the oral cavity and intestinal tract of dogs and cats and recovered from dog and cat bites in humans
P. stomatis	Dogs, cats	Isolated from the respiratory tract but not usually pathogenic
P. caballi	Horses	Respiratory infections including pneumonia
P. aerogenes	Pigs (man)	Commensal in intestine of pigs. Rarely pathogenic but one isolate associated with abortion. Abscesses from pig bites in humans
P. anatipestifer	Ducklings mainly but also chickens, turkeys, pheasants, water fowl	'New duck disease': severe fibrinous polyserositis in ducklings 1–8 weeks old
P. anatis	Ducks	Recovered from intestines. Pathogenicity not demonstrated
P. gallinarum	Poultry	Commensal in respiratory mucosa. Occasional low grade infections
P. avium, *P. langaa* *P. volantium*	Chickens	Isolated from the respiratory tract of healthy birds Pathogenicity not demonstrated for any of the three species

Capsular typing:
rapid slide agglutination, indirect haemagglutination for‘capsular’ typing using sheep red blood cells coated with bacterial extracts,
‘somatic’ typing by agar gel immunodiffusion tests using heat-treated cell extracts, or agglutination using acid-treated cells.
Confirmation of the isolates can be made using molecular techniques

One property of HS-causing strains of *P. multocida* is the ability to produce the enzyme **hyaluronidase** (13).

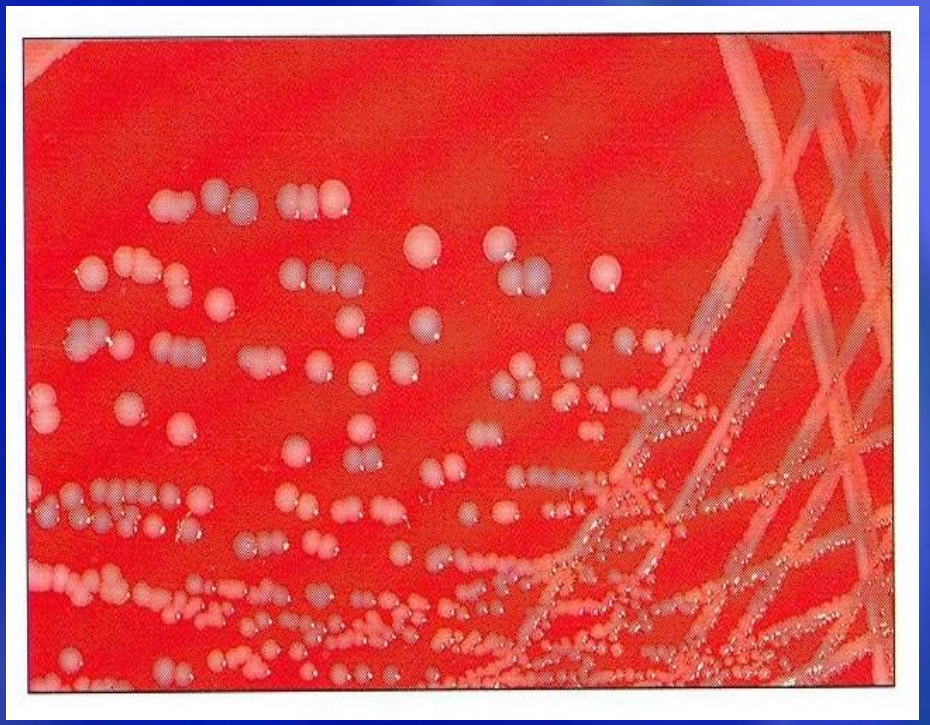
Having identified the genus and species by cultural characteristics and biochemical tests, hyaluronidase production may then be used as a specific test for HS-causing pasteurellae. It should be noted that B serotypes other than B:2 (or 6:B), and type E, are hyaluronidase negative

Identification

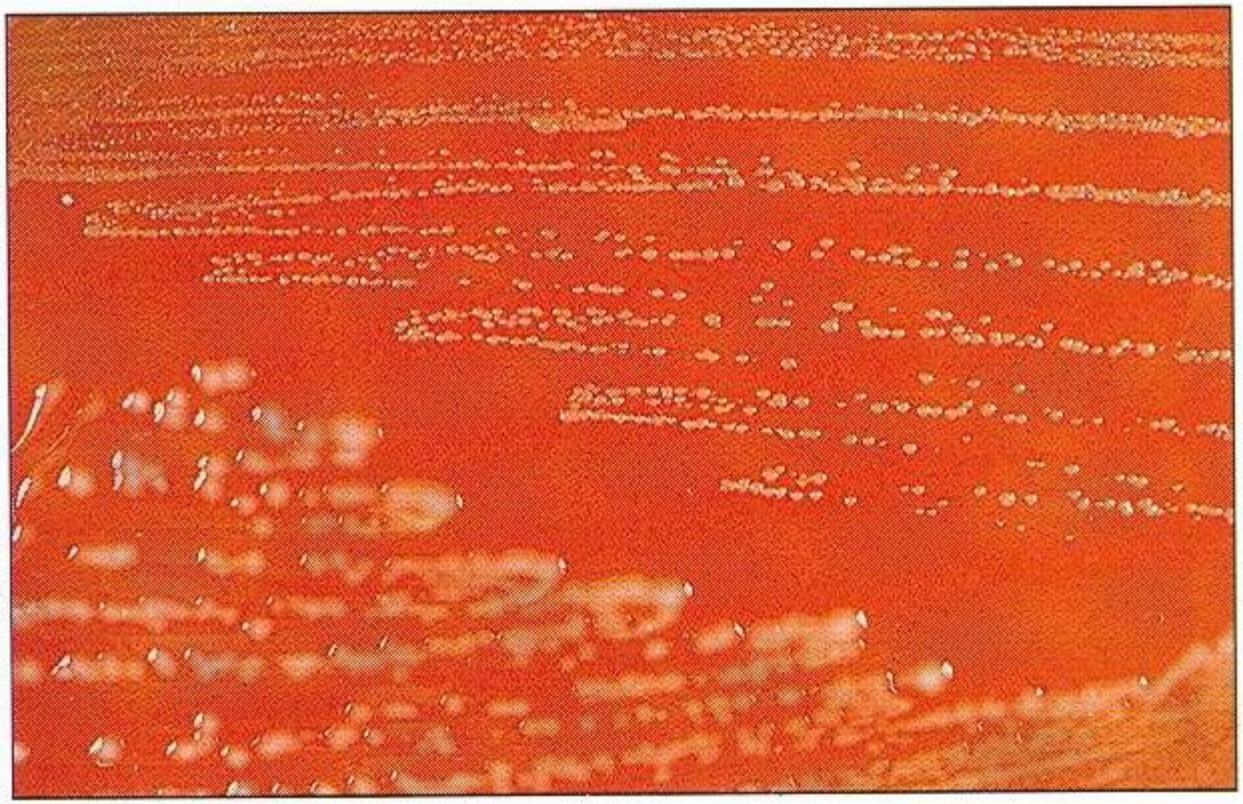
- **Colonial morphology-**
- **Colonies become evident in 24 hrs**
- **They are of moderate size, round and greyish.**
- **P. anatipestifer produce small dew drop colonies within 48 hrs.**
- **Colonies of *P. pneumotropica* are non haemolytic**
- **Type A strain of P. multocida often produce relatively large, mucoid colonies due to large capsules of hyaluronic acid**
- **P. multocida has a characteristic sweetish odour, is haemolytic, does not grow on MacConkey, good indole producer**

- HS organisms produce oxidase, catalase and indole, and will reduce nitrates. They do not produce hydrogen sulphide or urease, and fail to use citrate or liquefy gelatin.
- Glucose and sucrose are always fermented with the production of acid only.
- Most strains also ferment sorbitol. Some strains ferment arabinose, xylose and maltose, whereas salicin and lactose are almost invariably not fermented

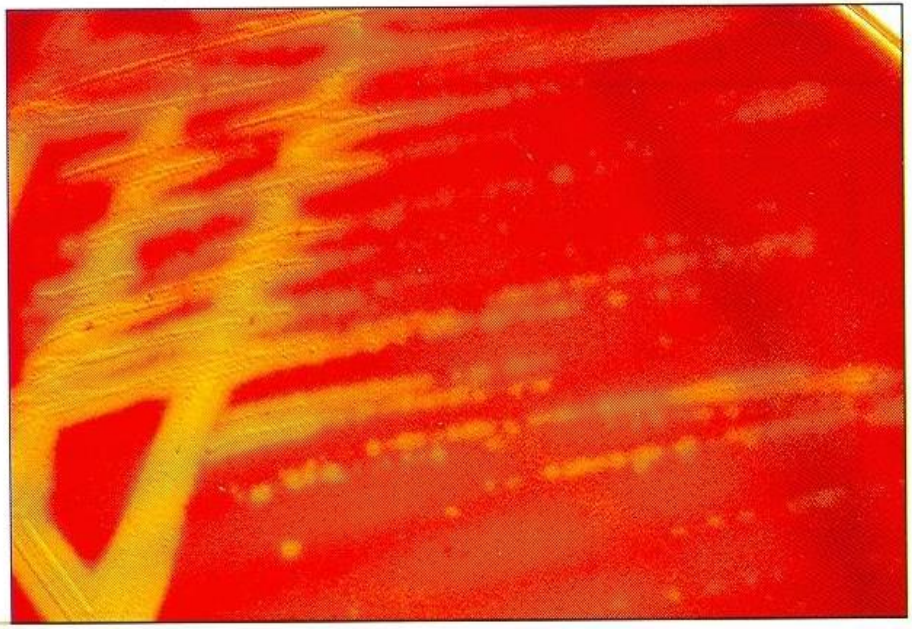
- P. haemolytica is haemolytic and bile salt tolerant in MacConkey
- Produce pink colonies
- No odour and does not produce indole
- **Microscopic appearance-**
- Smear from colonies reveal small, Gram negative rods or coccobacilli
- Pasteurella sp. are non-motile



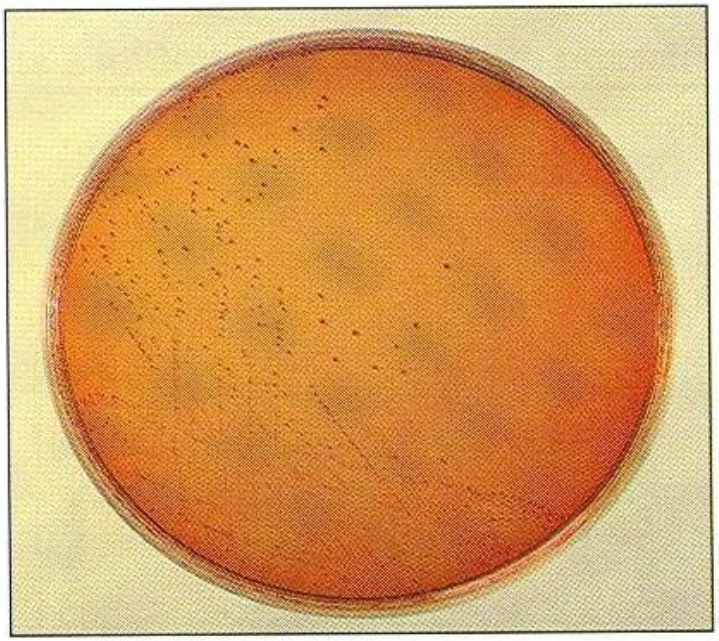
Pasteurella multocida on sheep blood agar. The colonies are non-haemolytic, and have characteristic sweetish odour



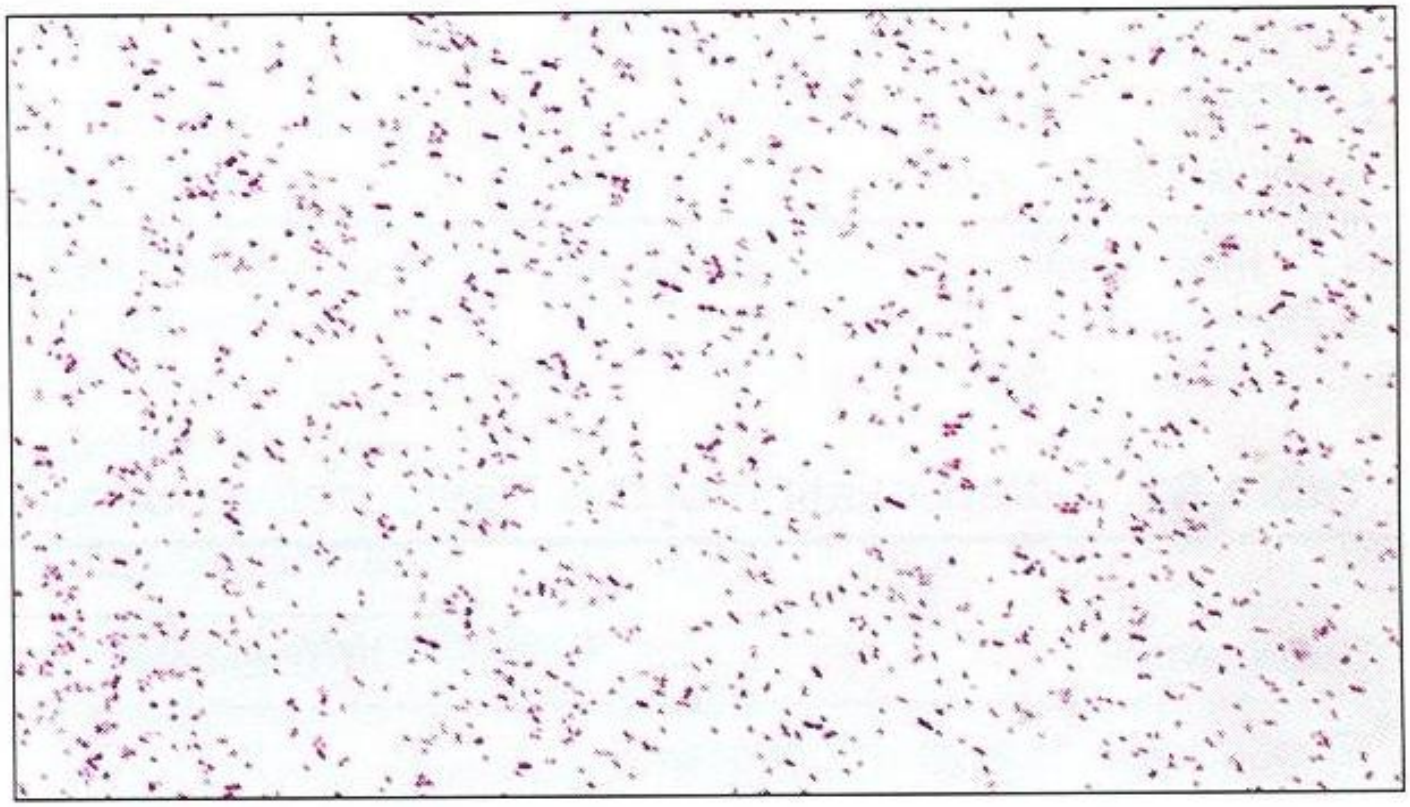
Comparison of the colonial types of *P. multocida*. The non-mucoid strain (top) of low virulence was isolated from a dog, while the mucoid colonies (bottom), are those of a virulent type A strain from a pig.



P. haemolytica on sheep blood agar isolated from the pneumonic lung of a lamb showing small colonies surrounded by a narrow zone of beta-haemolysis.



P. haemolytica on MacConkey agar: the small, red, pin-point colonies indicate a tolerance of the bile salts in the medium.



Gram-stained smear from a culture of *P. multocida*. Small Gram-negative rods with a tendency towards coccobacillary forms. (×1000)

Biochemical reactions

- Characteristic colonies yield small, Gram negative rods or coccobacilli that are oxidase positive and catalase positive

- On TSI-yellow slant and butt with no gas or hydrogen sulphide production

Differentiation of important *Pasteurella*

Species	Beta-haemolysis	Growth on MacConkey agar	Indole	Urease	Ornithine decarboxylase	Acid (24–48 hrs) from: Glucose	Lactose	Sucrose	Maltose	Mannitol
P. multocida	–	–	+	–	(+)	+	(–)	+	(–)	(+)
P. haemolytica	+	(+)	–	–	(–)	+	(+)	+	+	+
P. pneumo - tropica	–	(–)	(+)	+	+	+	(+)	+	+	–
P. canis	–	–	+/–	–	+	+	NA	NA	–	–
P. dagmatis	–	–	+	+	–	+*	–	–	+	–
P. caballi	–	–	–	–	+/–	+*	+	NA	(+)	+
P. aerogenes	–	+	–	+	(+)	+*	(–)	+	+	–
P. gallinarum	–	(–)	–	–	+/–	+	–	+	+	–

(+) = most positive; (–) = most negative; +/– = positive and negative strains; NA = data not available; +* = gas and acid from glucose.

Identification of the proposed subtypes of *P. multocida*

- *P. multocida ssp. multocida*: strains that cause significant disease in domestic animals
- *P. multocida ssp. septica*: strains recovered from various sources include dogs, cats, birds and man
- *P. multocida ssp. gallicida*: strains recovered from birds that may occasionally cause fowl cholera

Differentiation of various subtypes of Pasteurella multocida subtypes on the basis of fermentation of variious sugars

Subspecies	Fermentation of				
	Trehalose	D-Xylose	L-Arabinose	Sorbitol	Dulcitol
P. multocida subsp. *multocida*	v	v	–	+	–
P. multocida subsp. *septica*	+	+	–	–	–
P. multocida subsp. *gallicida*	–	+	v	+	+

v = variable reactions.

Mannheimia haemolytica

- Formerly called as *Pasteurella haemolytica*, biotype A
- Produces cytotoxin that kills alveolar macrophages and other leukocytes
- *Mannheimia haemolytica* causes pneumonia in cattle, goats, and sheep
- Other important disease include mastitis in ewes and septicaemia of nursing

Laboratory diagnosis

- Direct examination is of little use
- Media containing serum or blood are required for good growth of both species.
- Colonies are round, grayish and usually somewhat smaller than those of *P. multocida*
- Colonies surrounded by zone of β haemolysis
- Smears disclose Gram negative rods or coccobacilli
- They are indole –ve, non motile, and grow on MacConkey