CHAPTER II

REVIEW OF THE RELEVANT LITERATURES

1. Description of *Pasteurella multocida*

1.1 Taxonomy

P. multocida has been given many names according to the host animals. The isolates from cattle were called boviseptica; from pigs, suiseptica; from poultry, aviseptica and so on. After several name changes, Rosenbach and Merchant 1939 (41) proposed the name *P. multocida.* Later in Bergey's Manual of Systemic Bacteriology (42) the genus Pasteurella can be divided among six species with differential characteristics (Table 1) based on the beta hemolysis, growth on MacConkey agar, indole production, urease activity, gas from carbohydrates and acid production from lactose or mannitol (42). These species are *P. multocida, P. haemolytica, P. pneumotropica, P. ureae, P. aerogenes* and *P. gallinarum.*

1.2 Morphology, Growth Characteristics and Biochemical Reactions

P. multocida (43) is a small coccoid rod gram negative bacterium,0.25 μ-0.4 μ by 0.6 μ-2.6 μ in size. They are stained more distinctly at either pole, giving

Table 1. Differential characteristic of the species of the genus Pasteurella[a]

Characteristic	*P. multocida*	*P. pneumotropica*	*P. haemolytica*	*P. ureae*	*P. aerogenes*	*P. gallinarum*
Hemolysis(β)	-	-	+	-	-	-
Growth on MacConkey's agar	-	-	+	-	+	-
Indole production	+	+	+	-	-	-
Urease activity	-	+	-	+	+	-
Gas from carbohydrates	-	-	-	-	+	-
Acid production from:						
Lactose	-	d	d	-	-	-
Mannitol	+b	-	+	+	-	-

a Data from Carter (1981). For symbols see standard definitions

b Strains from dogs and cats may be negative for mannitol

rise to the term bipolar. Bipolar staining is common especially in preparation made from blood and infected animal tissue. They are generally capsulated, but non-capsulated forms have been reported. The majority of the species are non motile and non spore forming. After repeated culture on agar, the bacteria tend to form longer rods and to become more pleomorphic, forming chains, filaments and rods of various sizes. When grow in carbohydrate media for prolonged periods, marked pleomorphicity is noted. The organism usually possesses a capsule, it is mostly composed of hyaluronic acid (44). The bacterium is an aerobe and facultative anaerobe. Its optimum temperature for growth is at 37°c, and the maximum growth is reached in 18 hr in fermentor or shaker condition (45). The pH growth range is from 6-8.5 with an optimum of 7.2-7.4. The use of digested protein media or proteose peptone stimulate the growth of the organism. Bacteria can be grown in beef infusion media, but better growth is obtained when blood or serum is added to the media. Some strains fail to grow in media not containing blood or serum. Three principle colony types are seen on clear screen agar : fluorescent (smooth colonies), intermediate (mucoid colonies) and blue colonies (rough or non-capsulated colonies) (46). The fluorescent colonies are moderate in size, whitish, opaque, generally unstable and pathogenic. The mucoid colonies vary in appearance between fluorescent and the blue form. The blue colonies, although not necessarily rough in appearance, correspond in many ways to the R forms to other species. They are smaller, dewdrop-like and of a relatively low virulence. Strains giving rise to blue colonies are most frequently recovered from chronic infections. While acute

infections usually yielded strains giving rise to fluorescent colonies. Many group A strain produced mucoid variants on initial isolation on enriched media (Carter, 1985).

P. multocida is non acid-fast, non-spore forming and non-motile bacterium. The biochemical tests show catalase positive, always oxidase positive and MR-VP negative. Lysine decarboxylase and arginine dehydrolase are not produced. Ornithine decarboxylase is always positive and the gas is never produced. Strain of this species show great variation in their fermentation of carbohydrates. *P. multocida* ferments glucose, saccharose, mannose, and mannitol. Variable results are obtained with lactose, xylose, arabinose, dulcitol, sorbitol and fructose. Those substrates that are not usually hydrolysed are : raffinose, trehalose, rhamnose, inositol, salacin, dextrin and starch. It does not liquefy gelatin and only the lactose fermenting strains alter the indicator litmus milk. The hydrogen sulfide is not usually detectable in lead acetate agar or triple sugar iron agar but can be demonstrated if lead acetate paper is suspended over these media during incubation. Urease is not produced, although it is reported that some feline strains produce this enzyme.

1.3 Serotyping

The classification that gained widespread acceptance was based on serological properties of the organisms (47), capsular and cell wall compositions (48). Early attempts at serological classification date back to the 1920s (Table 2).

Table 2. Early attempts at classification of *P. multocida* (47)

Authors	Tests	Classification
Cornelius (1929)	Agglutination	Groups I, II, III, IV
Yusef (1935)	Precipitation	Groups I, II, III, IV
Rosenbuch and Merchant	Agglutination Fermentation	Groups I, II, III
Little and Lyon (1943)	Slide agglutination	Types 1, 2, 3
Roberts (1947)	Passive mouse protection	Types I, II, III, IV

In Carter, typing using precipitin tests (49) and indirect haemagglutination tests (50), four serological types were recognised on the basis of difference in capsular substances, designated A, B, C and D. In Carter 1961, a new serotype E was added and in 1963, type C was deleted.

A new capsular type F was added by Rimler and Rhoades, 1987 (51). This typing system was based on the specificity of the surface LPS and was commonly refered to capsular typing (Table 3).

In Namioka and Murata typing 1961 (52), using an agglutination tests with hydrochloride-treated cells, 11 somatic serotypes were recognised (52,53,54).

In Heddleston typing 1972 (55) using an agar precipitin tests, 16 somatic serotypes were recognised. This system used chicken antisera made against *P. multocida* bacterins which reacted to heat-stable antigens from formalinized-saline suspended bacteria (Table 3).

At the present time, *P. multocida* have been identified as 5 capsular types designated A, B, D, E and F (50, 51) and 16 somatic types. The popular method of designating serotypes is combined both capsular and somatic types (Table 4,5).

- Carter's system of capsular typing and Heddleston's system of somatic typing
- Carter's system and Namioka's system of somatic typing

The former serotyping system is identified by a capital letter representing the capsular type, followed by Arabic numerals 1 through 16 representing the somatic types. The important serotypes which are the cause of fowl cholera are 5:A, 8:A and 9:A and of hemorrhagic septicemia are 6:B (Southeast Asia) and 6:E (Central Africa).

Table 3. Current systems of classification of *P. multocida* (47)

Authors	Tests	Classification
Capsular		
Carter (1955)	IHA	Types A, B, C, D
Carter (1961)	IHA	Type E
Carter (1963)	IHA	Excluded type C
Namioka and Murata (1961)	Slide agglutination	Types A, B, C, D
Rimler and Rhoades (1987)	IHA	Type F
Somatic		
Namioka and Murata (1961)	Agglutination HCl treated cell	Types 1-11
Namioka and Bruner (1963)	Agglutination HCl treated cell	Types 1-11
Namioka and Murata (1964)	Agglutination HCl treated cell	Types 1-11
Heddleston, et al. (1972)	AGPT	Types 1-16

AGPT = Agar gel precipitin test

IHA = Indirect haemagglutination test

Table 4. Designation of serotypes of *P. multocida* by the Carter-Heddleston method

Capsular type	Somatic type	Serotype	Disease
A	1, 3, 4	A:1	Fowl cholera
		A:3	
		A:4	
	5, 6	A:5	Fowl cholera
		A:6	(Less common)
	7-10	A:7, A:8, A:9	
	12-15	A:10, A:12, A:13	
		A:14, A:15	
	16	A:16	Fowl cholera (turkeys)
B	2	B:2	Hemorrhagic septicemia
D	11	D:11	Atrophic rhinitis
E	2	E:2	Hemorrhagic septicemia

Table 5. Designation of serotypes of *P. multocida* by the Namioka-Carter method

Capsular type	Somatic type	Serotype	Disease
A	1, 3, 5, 7, 9	1:A	Pneumonia
		3:A	Pneumonia
		5:A	Fowl cholera
		7:A	Septicemia
		8:A, 9:A	Fowl cholera
B	6, 11	6:B	Hemorrhagic septicemia
		11:B	Wound infection
D	1, 2, 3, 4, 10	1:D, 2:D and 10:D	Pneumonia
		3:D	Pneumonia
		4:D	Pneumonia
E	6	6:E	Hemorrhagic septicemia

1.4 Antigenic Structure

The structure and cellular components of *P. multocida* may contribute to disease or stimulate host immune response (56). The major antigenic components are given below.

Capsules

The antigenic specificity of the capsular determines its serogroup. Capsular type A is compose of hyaluronic acid, which may act to mimic host antigens because hyaluronic acid naturally present in host tissue (57). Intimate associated with its other polysaccharides, proteins and lipids (1). The hyaluronic acid dose not exert antiphagocytic activity, but saline-extractable capsule material contained a factor capable of inhibiting the function of bovine polymorphonuclear leucocyte (58). The capsule of avian strains provided production from the action of complement (59). Removal of the hyaluronic acid capsule increased both the adhesiveness of the organism to animal cell surfaces (60) and its susceptibility to phagocytosis (61). The production of capsular material was affected by subminimal inhibitory concentration of antibiotics (62). Capsule was considered virulence factor because non encapsulated variants of pathogenic strains were less virulent than encapsulated forms (10).

Lipopolysaccharides (LPS)

Examination of strains from a variety of animal sources confirmed that LPS from *P. multocida* was similar to semirough LPS of Enterobacteriaceae (63). The LPS contained lipid, 2-keto-3-deoxyoctanate (KDO), heptose, glucose and

polysaccharide. The LPS of rabbit isolates contained either a non-serospecific antigen (R-LPS), a serospecific antigen (S-LPS) or both, from avian strains found to be R-LPS. The LPS responsible for antigenic specificity of somatic serotype (64), and when examined electrophoretically the molecular weight of LPS was low.

Toxins

The production of protein toxin by *P. multocida* has been recognised for sometimes although it is only in the last decade that some definition has been given to the nature of these products, following the discovery of toxigenic strain. Some strains, particular those of capsular type D (65) produced a factor designated dermonecrotic toxin (DNT). Purified DNT was a protein estimated molecular weight ranging from 112-160 kDa (66). Crude toxin was inactivated by formaldehyde, heat and proteolytic enzyme suggesting it was a protein (67). Commercial vaccines therefore incorporate formaldehyde treated whole cell of toxigenic *P. multocida* or formaldehyde-detoxified crude bacterial extracts of toxigenic organism (68). Strains from other hosts also produced the toxin including poultry, calves, cats and dogs (69), rabbits (70) and human respiratory tract (71).

Plasmids

Avian strains contained plasmids and this characteristic together with a degree of complement resistant were correlated to virulence marker (72). Plasmids confering antibiotic resistance have been isolated from fowl cholera and bovine pneumonia strains.

2. Disease Caused by *P. multocida*

The important diseases caused by *P. multocida* are fowl cholera in avian species and hemorrhagic septicemia in cattle (Table 4,5).

2.1 Fowl Cholera

Fowl cholera is a major disease problem in poultry e.g., chickens, turkeys, geese, ducks and many wild and zoologic birds. The important serotypes causing disease are 5:A, 8:A and 9:A. Usually the disease appears as an acute septicemia associated with high morbidity and mortality, but chronic condition also occurs. With acute cholera suddenly unexpected deaths occurs in the flock, mortality often increase rapidly. Sick birds show anorexia, depression, nasal or oral discharge of mucus, white watery or green mucoid diarrhea. The course of illness is short and often followed by death. The chronic cases, there often is swelling of a jont, wattle, footpad on tendon sheath. In chickens and turkeys, cholera is seen peracute, acute and chronic forms. The less spectacular, but still destructive chronic type disease is more frequently seen in some areas than the peracute form. Sporadic secondary infections of fowl are frequently found in the airsacs and sinuses. In addition to chickens, serious losses occur in domestic duck and geese.

2.2 Hemorrhagic Septicemia

Hemorrhagic septicemia (73) is one of the major disease caused by *P. multocida* serotype 6:B and 6:E, which is endemic in most part of tropical Asia, Africa and India. Cattle and buffalo are the usual hosts, although pigs, sheep, goats and camels are all susceptible. Hemorrhagic septicemia occurs in either the pectoral or edematous forms. The pectoral form involves the lung and the plural cavity and is accompanied by petechial hemorrhages in those tissue. The edematous type appears as an extensive edema of the subcutaneous tissue and the organs and tissues of the peritoneal cavity.

2.3 Human Infections

The most common form of pasteurella infection in human is that of animal bite-wound infections (47). Dog and cat frequently harbour pasteurella as commensals. Infected bite wounds can lead to septicemia and localization at certain site. A chronic respiratory tract infection has also been recorded in man. Most rarely, nervous system infection, peritonitis, appendicitis and urogenital infection are recorded. *P. multocida* often acts as an opportunistic pathogen and causes bacteremia in patients with liver dysfunction, septic arthritis in damaged joints meningitis in the very young and olderly patients and pulmonary colonization or invasion in patients with underlying respiratory tract abnormalities. The infection reported usually have been localized and chronic, indicating that man posseses considerable resistance to the

organisms. It is well known that dog and cat bite in man frequently result in infections with *P. multocida*.

2.4 Pathogenesis and Epidemiology

P. multocida is pathogenic for a large number of different animals. The organism varies in virulence from the highly virulent to the comparatively avilulent types. The virulence can be increased by animal passages, chicken embryo passages and growth upon blood or serum media. Spontaneous dissociated change which occurred in cultures also contributed to the loss of virulence in some strains. *P. multocida* plays as a secondary invader when the resistance of the animal has been reduced by various stress. The more susceptible of the experimental animals are the mouse, rabbit and pigeon. Guinea pigs are more resistant. Chickens are more susceptible to fowl cholera strains, although highly virulent ovine and bovine strains are able to produce death.

In natural infections, the portal of entry is probably the pharynx and / or upper respiratory tract. The acute infections are characterized by a septicemia, it invades to the bloodstream early after colonization. Frequently accompanied by blood-vascular congestion, submucous and subserous hemorrhages and enteritis. The less acute form of the disease is accompanied by serofibrinous or hemorrhagic lesions. The chronic type is characterized by necrotic areas, abscesses and the accompanying debilitative conditions, such as anemia, diarrhea and cachexia.

P. multocida is encountered throughout the world and is frequently the cause of great economic loss (43). Since typical organisms have been isolated from respiratory and digestive tracts of normal animals, the primary relationship of the organisms to the disease had been doubted frequently. These organisms may act as secondary invaders to other diseases or to debilitative predisposing factors such as several weather, long truck or train rides and faulty nutrition. The occurrence of epizootic and sporadic outbreaks, especially of fowl cholera, may indicate that these organisms can acquire virulence while being harbored in the animal, since the nature of these organisms requires continuing parasitism for existence. The organism is transmitted by contact and by consumption of contaminated food and water. In some case droplet infection is of significance.

3. Prevention and Control

P. multocida is the most prominent pathogen in domestic animals causing severe disease and major economic losses. Mechanism of immunity to these bacteria have been difficult to determine, and good vaccines have been developed and evaluated (74).

Currently, vaccination programs are neccessary to prevent disease. The types of vaccines are killed whole cell vaccine and live avirulent vaccine.

3.1 Hemorrhagic Septicemia Vaccine

Oil adjuvant vaccine is widely used and is generally credited with reducing the prevalence and incidence of hemorrhagic septicemia. Despite this significant outbreaks still occur in most endemic regions (75). Formalin fixed whole cell bacteria are generally used during an outbreak to protect animal at risk and have been thought to reduce further disease spread and stock losses. The protective immune response to hemorrhagic septicemia vaccination has long been attributed to humoral response as protection can be transfered to naive animals with serum from vaccinated animals (75,76).

3.2 Fowl Cholera Vaccine

To control and eliminate pasteurellosis is to develop an effective vaccine. Since Pasteur developed the first fowl cholera vaccine, there have many attempts to produce efficient vaccines against disease.

Killed Vaccine (Bacterins)

There are many types of killed vaccines used to prevent disease. Vaccines are usually prepared in formalinized saline solution. Bacteria prepared from tissue of turkeys that died of acute fowl cholera (7) and embryonating turkey egg (77) induced immunity in turkeys against infection with a different immunogenic type. The phenol-killed vaccine (4) was found to be effective in controlling duck cholera. The bacteria are incorporated with adjuvant to improve vaccine efficacy. Immunity

induced by this vaccine is type specific. In Thailand, the formalinized vaccine prepared from serotype 8:A local strain is used. The efficacy of this vaccine is 70% dependent on the virulence of an outbreak. Killed vaccines prepared from tissue of infected turkey induce immunity against different immunogenic types , but the bacteria prepared with bacteria grown on conventional agar media does not induce cross-immunity. These studies indicated that *P. multocida* produce wide spectrum of immunogens in vivo than in vitro (56,78, 79, 80).

Live Vaccine

Live vaccine are generally thought to provide higher protection against different serotypes than bacterins. Several live vaccines developed for vaccine efficacy improvement have been reported. The one of live avirulent vaccine was the Clemson University (CU) strain (13), serotype 3:A, 4:A (81). The CU strain was found to provide both humoral and cell mediated immunity (82), and to be protective against the major type that infected turkey (13). Although the CU strain stimulated an effective immune response in turkey, disadvantage of this was the resulting in mortality following vaccination (81, 83, 84).

The live avirulent streptomycin dependent mutant strain; P-1059 str-d (24) and temperature sensitive mutant strain; M3G (20), PM#1 and PM#3 (21) were mutagenized from live vaccine, *P. multocida* from dead turkey and CU strain treated with N-methyl-N′-nitro-N-nitrosoguanidine (84) respectively. The live mutant strains protected turkeys against experimental fowl cholera infection.

For prevention of disease may depend on a variety of environmental and management factors, host immunity and therapeutic protocols (85).

4. Protective Antigens of *P. multocida*

Prevention of disease by using killed and live vaccine can induce protective immunity, but sometimes the results in mortality, morbidity and systemic infection are important problem. For these reasons, many researchers attempt to an improved vaccine efficacy by the determination of the immunogenic antigens or subcellular materials. There are numerous reports of studies in which vaccine have been prepared by extraction of antigens.

Guant et al 1977 (29) reported an antigen extracted from *P. multocida* type A by an aqueous solution of 0.5 M KSCN in 0.08 M sodium chloride was found to be immunogenic in chicken against homologous and heterologous challenge.

Lu et al. 1987 (86) demonstrated that a KSCN antigen extract prepared from a virulent serotype 3:A contained protein, carbohydrate, LPS, DNA and RNA was safe and protected rabbit against homologous challenge. Furthermore, rabbit hyperimmune serum directed against the KSCN antigen extract also protect rabbits against homologous challenge, indicating that vaccine stimulated protective antibodies.

Subcellular fraction from 2.5% sodium chloride solution contained capsular antigen (34) and complex substance with high molecular weight, 40% protein and 15% carbohydrate (28). These capsular extract was immunogenic, all of turkey inoculated with this antigen survived from challenge.

The immunogenic role of OMP has been reported, Truscott et al., 1988 (36) demonstrated that 50 kDa OMP was antiphagocytic and turkeys treated with antibodies specific to the 50 kDa OMP were protected against challenge (36).

Lu et al.,1988 (87) identified protein immunogens of OMP by radioimmunoprecipitation (RIP) and Western blot analysis. They demonstrated that rabbits mounted major antibody response against 27, 37.5, 49.5, 58.7 and 64.4 kDa OMP. They further demonstrated that vaccination with OMP protected rabbits against homologous challenge (35). More specifically, MAb against 37.5 kDa OMP protected both mice and rabbits against challenge (37). Examination of OMP by SDS-PAGE were useful in analysis of OMP profiles (11) and to epidemiology and virulent markers (88).

P. multocida LPS has similar chemical and biological properties to the R-type LPS of the gram negative bacteria (89). Purified LPS is antigenic, however the level of antibody response following immunization depends on animal species, inoculated dose, LPS type, route and method of inoculation used (90). The role of LPS as an immunogen in mammals remains controversial. Mice, cattle and rabbits have been readily protected against infection following immunization with LPS (38).