Chapter 2

Review of the Relevant Literature

1. Description of Pasteurella multocida

1.1 Taxonomy

P. multocida was classified as belonging to the family Brucellaceae, genus Pasteurella (11), but it was listed under genera of uncertain affiliation in the 7th edition of Bergey's Manual in 1974 (12). Later, in Bergey's Manual of Systemic Bacteriology, 1984 P.multocida was classified as belonging to the family Pasteurellaceae, Genus Pasteurella which includes : P.multocida, P.haemolytica, P. pneumotropica, P.ureae, P.aerogenes and P.gallinarum (13) see table 1

1.2 Morphology,growth characteristics and biochemical reactions

P. multocida is a small gram-negative rod or coccobacillus. Many cultures are quite pleomorphic after repeat subculturing or when are grown under unfavorable conditions. It's size is about 0.2-0.4 um in width by 0.6-2.5 um in length (14,15). It generally shows bipolar staining when seen in stained films, in that the ends of the

Table 1 Differential characteristics of the species of the genus Pasteurella [a]

Characteristics	1.P. multocida	2.P. pneumotropica	3.P. haemolytica	4.P. ureae	5.P. aerogenes	6.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.

rods are more deeply stained than the central portion. It is non-sporeforming,non-haemohytic and non-motile.The organisms occur singly, in pairs,rarely in chains or in filament forms (11,15,16).

Many strains of P.multocida form a capsule when they are freshly isolated, but their capsules are lost quickly. The encapsulated strains are usually highly virulent whereas uncapsulated isolates are typically of low virulence (11,16,17). The capsules of the organism are large and are mostly composed of hyaruloronic acid (15).

P.multocida can grow aerobically or as a facultative anaerobe. The optimal growth temperature is 37°C; the temperature range for growth is 22 to 44°C.The optimal pH range is 7.2-7.8; but it is able to grow over the range of 6.2-9.0, depending on the composition of the medium (13,15,18).

Maximum growth is obtained at 16-24 hr in liquid media. This growth is enhanced when the medium is enriched with enzymatic digests of peptone,casein hydrolysate or avian serum. Excellent growth is obtained on 5% blood agar plates. The use of CO_2 during incubation may be helpful (11,15,18). The cultures have a distinctive odor, variously described as being musty, like semen, or like burning hair (18). On agar plates, three different colonial forms were recognized i.e. (a) smooth colonies or fluorescent colonies (b) mucoid colonies (c) rough or blue colonies or non-capsulated ones. The

smooth colonies possess well-developed capsules whereas mucoid colonies possess the mucoid antigen with or without the capsular antigen. In cultures of the rough type,capsular or mucoid antigen have yet to be demonstrated. The principal characteristics of these three varients are summarized in Table 2 (14,19).

Carter showed that the smooth or fluorescent strains possessed a type-specific capsular antigen by means of a precipitin reaction (20)

Carter and Bain (21) summarized the relationship between colonial variation and its virulence.e.g.,the smooth colonies are highly virulent in mice,the mucoid ones vary in their virulence,while rough colonies are low in virulence in the animal.

Some biochemical characteristics are described as following; it gives a positive reaction for oxidase and catalase but does not ferment citrate, nor does it liquify geltin.Other biochemical reactions are shown in Table 3 (22).

1.3 Typing of strains

In early literature, the strains designation depended on the type of animal from which they were isolated. Thus,the strain recovered from cows was called P.boviseptica,

Table 2. Some characteristics of the principal Variants of *Pasteurella multocida*

Variants	Colonies on agar	Growth in broth	Mouse virulence
Mucoid	Large, flowing	Mucoid deposit	Moderate
Smooth (fluorescent)	Medium sized, discrete	Diffuse	High
Rough (blue)	small, discrete	Autoagglutination	Low

Table 3 Biochemical characteristics of the species of the genus Pasteurella[a]

Characteristics	1.P. multocida	2.P. pneumotropica	3.P. haemolytica	4.P. ureae	5.P. aerogenes	6.P. gallinarum
Catalase test	+	+	+	+	+	+
Oxidase test (Kovacs')	[+]	+	+	+	+	+
Ornithine decarboxylase	[+]	+			+	[-]
Lysine decarboxylase	-	-			-	-
Arginine dihydrolase	-	-			-	-
H_2S production (lead acetate strip)	+	[+]			+	[+]
Nitrate reduced to nitrite	[+]	+	+		+	+
Growth in KCN	[+]	d				
Methyl red and Voges-Proskauer test	-	-	-	-	-	-
Acid production from:						
Adonitol	[-]	[-]	-			-
Amygdalin	[-]	d				
Arabinose	d[b]	[-]			+	-
Cellobiose		[-]				
Dextrin	d		+			+
Dulcitol	d[b]	[-]	d	[-]	-	-
Erythritol	[-]	[-]	-			
Esculin	[-]					
Fructose				+		+
Galactose	+	[+]				+
Glucose	+	[+]	+	+	+	+
Glycerol	d	[+][b]	d	[-]	+	-
Glycogen	[-]	[-]	+			
Inositol	[-]	d	+			-
Inulin	[-]		-			-
Maltose	d[c]	[+]	+		+	+
Mannose	+	[+]				+
Melezitose	[-]					
Melibiose	d		+			
Raffinose	d	d	+		-	d
Rhamnose	[-]	[-]	d		d	-
Salicin	[-]	[-]		[-]	-	
Sorbitol	[+][d]		+	+	-	d
Sorbose	[-]					
Starch		[+]	+			
Trehalose		[+]			-	+
Xylose	[+][d]	[-]		-	+	d

[a] Data compiled from Carter (1981), Ghosien et al. (1973) and Smith (1958,1974). Symbols: +, all strains positive: [+], most strains positive: d, differs among strains: [-], most strains negative: -, all strains negative.

[b] Arabinose and dulcitol fermentation is most common in strains from birds.

[c] Maltose fermentation is characteristic of strains from dogs and cats.

[d] Strains from cats and dogs may be negative for sorbitol and xylose.

the one from birds P.aviseptica and that from sheep P. oviseptica. However, this method of classification was not fully accepted at that time and there were reports of strains which had no serological relationship,being isolated from the same species of animal (23,24).

For several decades, the serologic features of P. multocida have been studied by many investigators such as Cornelius(25), Ochi (26) and Yusef (27). Unfortunately, none of these investigations was followed up. Several investigators and their works are listed in Table 4

Rosenbusch and Merchant (14,28) divided their strains into three principle groups,designated I,II, and III on the basis of fermentation of xylose, arabinose, and dulcitol. by means of a conventional agglutination procedure

Little and Lyon (29),employing a slide agglutination test, divided their strains into three serological types, designated type 1,2, and 3. Type 3 was the most prevalent (15)

Robert (30) has demonstrated four types of this organism, designated I,II,III and IV based on a serum protection test conducted on mice.

Carter (20) employed a precipitin test by using a saline extract of capsular material as an antigen and capsular swelling techniques. As a results he was able to divide

Table 4 Early Serologic Studies on Pasteurella multocida

Investigators	Procedure	Results
Cornelius (1929)	Agglutinin absorption	Groups I,II,III,and IV
Ochi (1933)	Agglutination	Types A,B,C,and D
Yusef (1935)	Precipitation	Groups I,II,III,and IV
Rosenbusch and Merchant (1939)	Agglutination, fermentation	Groups I,II,and III
Little and Lyon (1943)	Slide agglutination	Types 1,2,and 3
Roberts (1947)	Serum protection tests in mice	Types I,II,III,and IV

the organism into four types : A,B,C,and D.

Carter (28,31,32,33,34,) performed an indirect haemagglutination test involving the adsorption of a specific capsular substance onto erythrocytes. This test was quite sensitive, and four types - A,B,C,and D - were identified on the basis of differences in their capsular antigens. Subsequent research indicated that Type C was not of the capsular type; hence it was omitted.

Carter (35) recovered nine strains of P.multocida from cases of bovine hemorrhagic septicemia in Central Africa; these strains were identified as members of a new capsular type and designated as type E. There were, therefore; only four capsular types, namely A,B,D and E

Carter has shown that types B,A,C and D were identical with Robert's types I,II,III, and IV, and demonstrated a strong correlation between serological types and host species (28). Type B strains include most of those causing epizootic pasteurellosis or haemorrhagic septicaemia in cattle (36). Type A strains were frequently isolated from cases of fowl cholera (15).Type C cultures were seldom isolated. and at first they were believed to come from dogs and cats,but since this was not confirmed,the category was subsequently abandoned (21,37,38). Type D strains were associated with a wide range of infections in cattle, sheep, pigs and poultry, including for example, shipping fever in

cattle, Viral pneumonia of pigs,sporadic pneumonia in cattle and pigs, as well as fowl cholera and bovine mastitis (39).

Carter's serological classification of the Pasteurella organism was widely used internationally as being the most reliable system (23)

Carter & Bain (21) reported the capsular types of Pasteurella isolated from various types of animals as seen in Table 5. They stated that A and D were the most widely distributed geographically.

Some investigators, such as Namioka and Murata (40), Norung (14) described a simplified slide agglutination procedure as a substitute for the indirect haemagglutination test, which they claimed was less reliable.

It was clear from a number of investigations that there were diverse somatic antigens shared by many strain. To demonstrate these different somatic components, Namioka and Murata (23,41) removed the capsular material by treatment with 1N HCl. The treated cells were readily agglutinable and were subjected to a conventional tube agglutination procedure. Namioka and Murata established eleven O groups on the basis of their somatic antigen; later 15 O groups were found (42).

Bain considered that the material remaining after the HCl treatment was a part of the cell wall substance and

Table 5 Relation between serological type of P.multocida and animal host

Animal Host	Type (Capsular) A	B	D	Not typable
Buffalo	2	13	0	0
Caribou	3	0	0	0
Cat	3	0	1	27
Cattle	88	23	6	47
Chinchilla	0	0	0	7
Chipmunck	0	0	0	1
Deer	0	1	1	0
Dog	0	0	3	14
Fowl	56	0	6	125
Goat	0	0	1	1
Guinea pig	1	0	2	1
Horse	0	1	1	1
Man	20	0	11	16
Mink	3	0	1	10
Monkey	1	0	0	0
Mouse	0	0	2	3
Muskrat	1	0	0	2
Pig	157	3	118	19
Rabbit	11	0	3	13
Sheep	11	1	9	13
Totals	357	42	165	300

therefore, it was probably appropriate to call it a cell wall antigen (21).

It was assumed that the substance was a lipopolysaccharide combined with a protein (21).

Namioka and Murata (43) proposed that serotypes be identified by first listing the number standing for the specific somatic, or O component, followed by the capital letter standing for the specific capsular, or K-substance, for example: 5:A, 8:A

The complexity of the somatic components of some strains was shown by demonstrating a large number of O subgroups. Their relation to the different capsular types are listed in Table 6 (23).

As is evident from this table, the six O groups are in Carter's type A, two O groups are in type B, six O groups are in type D and one group is in type E.

Heddleston (44) pointed out that two strains of the organism (P-1059,70-x), which differ immunologically, cause fowl cholera. When two strains are serotyped, both strains belong to Carter's capsular type A but O group 8 is presented in P-1059 and O group 5 is presented in 70-X.

Table 6 Relation between Carter's capsule and o group

Carter's capsule group	O group
A	1
	3
	5*
	7
	8*
	9*
B	6**
	11
D	1
	2
	3
	4
	10
	12
E	6**

* Pathogen of fowl cholera

** Pathogen of hemorrhagic septicemia

The findings of Namioka and Bruner (42) with respect to avian serotypes are summarized in Table 7, 5:A and 8:A being the most prevalent

Carter collected about 150 strains by combining them with Carter's capsular antigens. These are divided into 15 serotypes, as shown in Table 8.

1.4 Antigenic Makeup and Toxin

The antigenic structure of P.multocida is complex (45). Although a number of investigators have studied the antigenic make up of many strains of P.multocida, it is difficult to interpret their results, because the different serological varieties have been studied by different methods. The occurrence of serologically active polysaccharides has been shown by several workers. Agar gel precipitin tests revealed at least 12 antigens in saline extracts of cells (14).

Carter and Annau (46) have analyzed a type-specific capsular substance extracted from cells suspended in water and heated at 56°C for 30 minutes and found them to be polysaccharides. The depolymerization of the polysaccharide by hyaluronidase has proven that it is at least hyarulonic acid in part.

The basic facts concerning the major antigenic components are given below. Most of the information is

Table 7 Avian Serotypes of P.multocida identifed by Namioka and Bruner.

Somatic group (O)	Capsular type (K)	Serotype	No.of strains	Origin
5	A	5:A	27	Chickens and turkeys
8	A	8:A	7	Turkeys
9	A	9:A	1	Turkey
2	D	2:D	4	Chickens
4	D	4:D	1	Chicken
?	D	-:D	1	Turkey

Table 8 Relation between Pasteurella serotypes of capsule and somatic and host animals

O group	Capsule	Sero-type	Process of disease	Animals	Strain examined
1	A	1:A	Pneumonica	Swine	9
			Septicemia	Mouse	2
	D	1:D	Pneumonica	Swine	1
	-*	1:-	Pneumonica	Swine	1
				Sheep	2
				Cattle	2
2	D	2:D	Pneumonica	Swine	12
3	A	3:A	Pneumonica	Swine	3
	D	3:D	Pneumonica	Cat	1
4	D	4:D	Pneumonica	Swine	2
			Pneumonica	Sheep	2
5	A	5:A	Fowl cholera	Fowl	13
			Pneumonica	Swine	1
	-	5:-	Fowl cholera	Fowl	21
			Pneumonia	Swine	1
			Local wound	Men	1
6	B	6:B	Hem. Sept.	Cattle	6
	E	6:E	Hem. Sept.	Cattle	1
	-	6:-	Hem. Sept.	Cattle	10
7	A	7:A	Septicemia	Cattle	5
	-	7:-	Septicemia	Cattle	2
8	A	8:A	Fowl cholera	Fowl	1
9	A	9:A	Fowl cholera	Fowl	7
10	D	10:D	Pneumonia	Swine	1
11	B	11:B	Local wound	Cattle	1

* No capsule was possessed.

Hem.Sept.:Hemorrhagic septicemia

based on the studies of Bain and Dhanda on type 1 strains (47,48).

Polysaccharides. These soluble substances are produced by,and easily recovered from,capsulated strains and are probably produced by some noncapsulated strains too. They behave as haptens and act as precipitating and complement fixing substance. Their capacity to elicit some protection in mice is thought to be due to the presence of protein.

Hyaluronic acid. This mucopolysaccharide is produced in large quantities by some type A strains and is largely concentrated in the capsule. In its purified state, it is nonantigenic

Lipopolysaccharides. These substances are employed in the indirect hemagglutination procedure and are responsible for "so-called" capsular specificity, although they are not necessarily confined to the capsule. They are toxic and pyrogenic in the case of rabbits and mice. They are strongly antigens and some preparations elicit immunity such as protective antibodies in rabbits which passively protect mice. Their precise immunologic behaviour probably depends on their purity.

Toxins. Endotoxin is produced by both virulent and non-virulent P.multocida. The difference between virulent

encapsulated and non-virulent, unencapsulated strains is not their ability to form endotoxin but the ability of virulent strains to survive and multiply so that sufficient endotoxin is produced in vivo, to give rise to pathologic processes (15).

This endotoxin is a nitrogen-containing phosphorylated lipopolysaccharide, readily inactivated under mild acid conditions. Signs of acute fowl cholera were induced in chickens by injection of fractional amounts of the endotoxin (15).

1.5 Diseases caused by P.multocida

The principle diseases and infections caused by P. multocida are cholera of domestic and wild fowl, hemorrhagic septicemia of cattle and water buffaloes, primary or secondary pneumonias of a number of species, and sporadic infections of a wide variety of animals (14). There is evidence that P.multocida exists as a commensal in respiratory tracts of animals, and that certain predisporing conditions alter this balance between host and parasite, resulting in the development of disease (49)

More frequently P.multocida acts as a secondary invader rather than being the primary cause of various diseases when it is present in the lungs of cattle and swine (14).

An important aspect of the epidemiology of pasteurellosis in animals is that the bacteria can be carried by individual cerature which show no outward signs of clinical infection. Since the organisms are relatively susceptible to chemical and physical agents, the carrier animal would seem to play an essential role in the life history of these bacteria and in their distribution from one host to another (15).

The development of disease from the carrier states, is related to predisposing conditions concerned with climate, methods of husbandry and other concurrent infections.

1.5.1 Fowl cholera

The pathogenicity or virulence of P.multocida in relation to fowl cholera is complex and quite variable depending on the strain of P.multocida and variations within the strain or host. The ability of P.multocida to invade and reproduce in the host is related to the capsule (15). According to Carter's study, many isolants from cases of fowl cholera have large capsules but are of low virulence. Therefore, he concluded that the ability to invade and grow within the host is apparently related to some chemical substance associated with the capsule rather than with its physical presence (50).

Fowl cholera occurs sporadically or

enzootically in most countries around the world (15). It is an infectious disease affecting poultry, waterfowl and domesticated birds, including, chickens, turkeys, ducks and geese. It usually appear as an acute septicemic disease with a high incidence of morbidity and a high mortality rate. Death may occur suddenly without the development of any early characteristic symptoms. The affected birds generally exhibit signs of depression, sleepiness and a lack of appetite. Breathing is rapid and done with the beak open, the feathers are ruffled. There may also be a yellowish diarrhoea (11,17)

A chronic, localized form also occurs in poultry and may follow the acute form or occur independently. The infection often results in the development of swollen wattles and comb, and sometimes the joints become hot and painful (49). Serotype 8:A is prevalent in Thailand. (51,52)

1.5.2 Hemorrhagic septicemia

One of the diseases cause by P.multocida is hemorrhagic septicemia. It is a disease of cattle, goat and sheep, usually taking the pectoral or pneumonic form. The affected animals suffer from a very high temperature. During the later stages of the disease they can breathe only with great difficulty. The disease often occurs after exposure or shipping (11)

1.6 Pathogenesis and Epidemiology

The pathogenicity of strains varies greatly, from highly virulent to moderately virulent fowl cholera,and acts as a secondary invaders and as commensals in the nasopharynx.

The pathogenic mechanism is not known but the available evidence indicates that a toxic component of the bacterial cell is responsible for the pathological effects and death is due to acute septicemias (53).

Epidemiological studies have revealed that the portal of entry of the organism is the pharynx and the upper respiratory tract (54).The organisms multiply in the lungs and are then disseminated to the liver and spleen,where they cause pathological changes. The terminal manifestations of the disease are similar to those observed during endotoxic shock. P.multocida usually enters the tissues of birds through the mucous membranes of the pharynx or upper air passages, but not through the esophagus, crop, or proventriculus (15).

1.7 Treatment

It is essential that treatment be initiated in the early stages of the disease if it is to be of value. Antibacterial chemotherapy has been used extensively in the

treatment of fowl cholera with varying degree of success, depending to a large extent on the promptness of treatment and the drug used. Sensitivity testing is often advantageous, since strains of P.multocida vary in susceptibility to chemotherapeutic agents and resistance to treatment may develop especially if the use of these agents is prolonged (55).

Sulfonamides

A number of sulfonamide compounds have been found effective in the treatment of infections. Sulfaquinoxaline is one of the most effective treatments so its use in the field is widespread. In amounts of 0.01-0.05% in drinking water, sulfaquinoxaline provides complete prophylactic in experimental fowl cholera when treatment is started 24 hours before the birds are inoculated (17,56). Most medication is given in the feed or water. Drugs in common use include : (17,57)

Sulfadimethoxine
Sulfaquinoxaline
Sulfamethazine
Sulfamerazine
Sulfaethoxypyridazine

Practically, medication may be administered together with the vaccine so as to stop the outbreak promptly.

Antibiotics

The tetracycline antibiotics have been found effective in a wide range of infections due to P.multocida and Hurt (58) successfully treated cholera in ducks with water-soluble erythromycin. Penicillin has been found to be of value in the treatment of Pasteurella infections both animals and humans (59). Chlortetracycline reduces losses in chicks about 80 % when given at the rate of 40 mg/kg body weight intramuscularly a half hour after parenteral inoculation of the organism (15).Stuart et al. (60) found that chlortetra-cycline activity against experimental fowl cholera is enhanced by the addition of 2% sodium sulfate to the diet. Streptomycin given intramuscularly in dosages of 150,000 ug prevents death when administered before or at the time of inoculation of P.multocida in adult turkeys (15).

An infected flock must be moved to clean premises or the sanitation improved to a marked degree during an outbreak. This may help slow down the development of cholera. Vaccination during the course of an outbreak is seldom effective. If cholera recurs repeated when treatment is discontinued, it may be necessary to depopulate the area of livestock (17).

1.8 Prevention and Control

Prevention of fowl cholera can be effected by

eliminating the reservoirs of P.multocida or by preventing their access to poultry flocks. Good management involves good sanitation, good ventilation of pens and the avoidance of overcrowding. Vaccination should be considered in areas where fowl cholera is prevalent, but it should not be seen as a substitute for good sanitary practices (15).

1.9 Type of fowl cholera vaccine used and available

Vaccination for the prevention of fowl cholera has been practiced widely for many years with irregular results. Some producers are attempting to up-grade or to improve their progeny by careful selection of strains and by greatly increasing the amount of antigen in each dose (14).

The Pasteur vaccine was designed to combat fowl cholera.It was made by Pasteur in 1880 and its success formed the basis for many other studies that laid the foundations for our knowledge of artificial immunization against infectious diseases. The Pasteur vaccine consisted of living cultures of two grades of virulence. It was administered as a prophylactic measure in two doses, given a few days apart, the first being less virulent than the second. This type of vaccine, however, is no longer used (11).

1.9.1 Killed whole-cell vaccines (Bacterin)

Killed whole-cell vaccines consist of

bacterins without adjuvant and bacterins with adjuvant.

1.9.1.1 Bacterins without adjuvant

These may be cultures of the specific organism killed by heat or chemicals or a suspension of formalinized chicken embryo. Since 1953 a controversy has been raging concerning the immunizing properties of chemically killed cultures of P.multocida for the control of fowl cholera (2).

1.9.1.1.1 Phenol-killed vaccine

This type of vaccine is made by the addition of 0.5 percent phenol. It is reported to be effective in controlling duck cholera (17).

1.9.1.1.2 Formalin-killed vaccine

Formalin is widely used to sterilize Pasteurella vaccines. It can bine to the protein and polysaccharide elements in extracts of Type B. It is cheap,effective, and a good preservative. The concentration in the vaccine itself may have to be as high as 0.5 percent, to inhibit the growth of molds (61). Bolin, Turn, and Eveleth (7) who first employed formalin to preserve cultures concluded that the highest degree of immunity was produced in about a week.This

type of vaccine, employed by many investigators, is a suspension of formalin-killed bacteria (3,4,62,63,64).

1.9.1.1.3 Chicken embryo vaccine

This was first used by Carter (90). Later, Dougherty modified the production of the egg embryo vaccine by inoculating with ten-day duck embryos which had been incubated for 24 hours with 0.1 ml each of allantoic fluid from third-passage eggs.Then the contents of each egg were added to 100 ml of normal saline solution and mixed for several minutes in a Waring Blender. Finally, formalin was added to make a final concentration of 0.25 percent. A comparison of the immunogenicity of seven P.multocida bacterin was performed. Dongherty found chicken embryo vaccine to be superior to all other bacterins (2).

1.9.1.2 Bacterins with adjuvant (adjuvanted Vaccines)

1.9.1.2.1 Alum-precipitated Vaccine

This is prepared very simply from broth bacterin. The method involves adding enough of a 10% potash alum solution to a formalinized suspension to give a concentration of 1 % alum in the vaccine (65). It represents one of the earliest adjuvanted vaccines.

1.9.1.2.2 Oil-adjuvanted vaccine

Adjuvants have been used to improve bacterins. Adjuvants are designed to retard absorption of the vaccine and prolong the antigenic stimuli to the antibody-forming cells. The use of light mineral oil in water-in-oil emulsified vaccines has been comprehensively reviewed by Freund (5). A method was described for the production of emulsified hemorrhagic septicemia vaccine.Although in Thailand,fowl cholera vaccine has never been used in this particular form. Attempts to develop adjuvanted vaccines concentrate on the use of formalin-killed Pasteurellae, suspended in stable water-in-oil emulsions. (8,9).

The bacterial suspension, killed by formalin, is emulsified in light mineral oil using a stabilizer of a type suitable for forming emulsions. There are three types of emulsions : the oil-in-water type, the water-in-oil type and the multiple emulsion type (65).

An emulsion is a system containing two liquid phases, one of which is dispersed as globules in the other. The liquid which is broken up into globules is termed the dispersed phase, whill the liquid surrounding the globules is known as the continuous phase. When one of the liquids is water and the other is oil, and it is refered

to as an oil-in-water type, this means that the oil is dispersed as globules in the water. A multiple emulsion type consists of either oil-in-water in oil (o/w/o) or water-in-oil in water (w/o/w).

Emulsions must be made with care so that the individual droplets which were suspended in the continuous phase are small enough (diameter 0.1-100 um.) and sufficiently stabilized to prevent coalescence (66,67).

1.9.2 Live vaccine

The killed vaccines, administered parenterally, are cumbersome to use, especially in large turkey flocks. They do not afford protection against heterologous serotypes. The live vaccine has the advantage of being easy to administer in drinking water and is found to afford protection against a wide spectrum of Pasteurella serotypes known to cause fowl cholera in Turkeys (68). The live vaccine, prepared from an attenuated strain or avirulent strain, helps reduce losses in affected flocks that cannot be saved by killed vaccines, However, the duration of immunity of live vaccine is no longer, and possibly shorter, than that obtained with adjuvanted vaccine (69,70).

In the United states live vaccines are based on the Clemson University strain of an attenuated P.

multocida.It is often used 2-4 weeks following inoculation with the oil-emulsified vaccine (17). Live oral vaccines for chicken and turkeys are available in many countries. These vaccines have been prepared with either the CU strain, the Heddleston strain, or the M2283 strain of P.multocida (71,72).