CHAPTER-I

**INTRODUCTION**

Agriculture is the backbone of economy of Bangladesh. About 21.8% of Gross Domestic Product (GDP) comes from agriculture sector of which Livestock alone contributes 2.50% (**BBS, 2011-2012**). Livestock sector is playing vital role in the National economy of Bangladesh. Increasing trends of production and supply of milk, meat and egg as animal protein from these sector is very important for the development of a meritorious and healthy nation. Government has given high priority on this expanding sectors and as a result huge number of livestock & poultry farms and poultry hatcheries have been established at govt. and private levels, which created a positive impact on socio-economic condition, food security, poverty reduction and employment generation. Moreover, this sector is labor intensive and quick-yielding sectors, which can make substantial contribution in augmenting growth and alleviating poverty. According to Bangladesh Economic Review 2011, the share of livestock sub-sector is 2.57 percent. Though the share of the livestock sector in GDP is small, it provides 90.00 percent of our daily protein requirements. Within the livestock sector, poultry farming has attained an important place through contribution to GDP and employment facility to urban and peri-urban people. Over the last two decades poultry farming has gradually taken the shape of a large industry and it is now one of the intensive forms of agri-business in our country. There are 11,24,29,000 chicken at backyard system (**Report of household based livestock and poultry survey, 2009**). In order to achieve Millennium Development Goal (MDG) Bangladesh is committed to lift up the poultry sector. Scientific breeding, feeding, management and disease control are the key point of success in poultry improvement program.

However, this sector is now facing many hazardous situations which are a great threat to poultry industry. A significant proportion of layer birds die due to diseases of which fowl cholera alone can cause a devastating mortality. The disease occurs sporadically & enzootically as per-acute, acute or chronic form all over the world (**Rimler & Glisson, 1997**) including Bangladesh (**Baki *et al*., 1991**). **Rashid *et al.,* (2013)** reported that 3.23% mortality is attributed by fowl cholera in commercial layer chickens of Bangladesh.

**Dutta** ***et al***., **(2013)** reported that 32.29% of indigenous chickens are died from fowl cholera in some selected areas of Rajshahi, Bangladesh.

**MZ Uddin (2011)** reported that fowl cholera is the cause of 0.46% mortality in ISA-Brown and Hy-Line strain of layer chicken reared in cage in Bangladesh.

In Bangladesh, Chittagong has an active and important role in poultry industry. Poultry production activity in Chittagong has a tremendous improvement in recent years. Due to the increasing poultry population, various diseases have become significant in Chittagong, of which fowl cholera is an economically important disease. Fowl cholera is caused by *Pasteurella multocida*, a heterogeneous bacterium that produces septicemia and/or respiratory disease in poultry. *P.multocida* was first found in 1878 in cholera-infected birds. However, it was not isolated until 1880 by Louis Pasteur- the man in whose honour Pasteurella is named (**L Pasteur, The attenuation of causal agent of fowl cholera**).

The disease generally occurs in epidemic form in a flock. It is often difficult to determine which of many possible sources of the causative agent *P. multocida* is responsible for an outbreak. The severity and incidence of *P. multocida* infections may vary considerably depending on several factors. No single virulence factor has been associated with the observed variation in virulence among strains. The epidemiology of fowl cholera appears complex. Traditional serotyping systems are only of limited use in epidemiological studies. In recent years, molecular typing methods have been applied to avian strains of *P. multocida* of different origin (**Christensen *et al.*, 1999)**. The results obtained using these newer methods indicate that wild birds may be a source of infection to commercial poultry (**Christensen *et al*., 1999)**. Carrier birds seem to play a major role in the transmission of cholera (**Rimler and Glisson, 1997)**. Surviving birds from diseased flocks appear to represent a risk, but more recent investigations indicate that carriers of *P. multocida* may exist within poultry flocks with no history of previous outbreaks of fowl cholera (**Muhairwa *et al.,* 2000)**. The site of infection for *P. multocida* is generally believed to be the respiratory tract (**Rimler and Glisson, 1997)**. The outcome of infections may range from per acute/acute infections to chronic infections. In the former type of infections, few clinical signs are observed before death and the lesions will be dominated by general septicemic lesions. In chronic forms of *P. multocida* infections, suppurative lesions may be widely distributed, often involving the respiratory tract, the conjunctiva and adjacent tissues of the head (**Gustafson *et al.,* 1998)**.

Diagnosis is always dependent upon isolation of the organism. For the detection of subclinical infections, mouse passage of relevant samples is recommended (**Muhairwa *et al.,* 2000)**, but polymerase chain reaction and isolation attempts on selective media may represent alternatives. Confinement is probably the most effective way to prevent introduction of *P. multocida.* However, extensive management systems dominate in many parts of the world, and under such circumstances vaccination is recommended as a preventive measure. Unfortunately, the development of safe and efficient live vaccines still poses problems. As a result, control remains dependent on bacterins which exhibit significant disadvantages compared to live vaccines.

Ever since Pasteur developed the first fowl cholera vaccine, many other live or inactivated products have been produced in an effort to control the disease. But vaccine developed from one serotype cannot give protection against other serotypes (**Heddleston and Watko, 1965**). For the development of a potent vaccine, knowledge of all the serotypes prevalent in and around Chittagong is very essential.

**Objectives:**

* To identify the *P. multocida* micro-organism from dead layer birds.
* To calculate apparent & true prevalence of fowl cholera in Chittagong.

CHAPTER-II

 **LITERATURE REVIEW**

Fowl cholera (avian cholera, avian pasteurellosis, avian hemorrhagic septicaemia) is an acute or chronic contagious disease affecting domestic as well as wide range of wild birds causing septicemic condition with high mortality in acute form. On the contrary, chronic form shows localized symptomatic infection. The chronic form is also known as “Wattle cholera”.

Fowl cholera is a contagious disease affecting poultry (**Dorsey, 1963 and Heddleston *et al*., 1972**).

**2.1 Etiology**:

The disease is caused by *Pasteurella* *multocida*. The scientific classification of the bacterium are given below:

Kingdom : Bacteria

 Phylum : Proteobacteria

 Class : Gamma Proteobacteria

 Order : Pasteurellales

 Family : Pasteurellaceae

 Genus : Pasteurella

 Species : *P. multocida*

 Three subspecies of *P*. *multocida* (*P*. *multocida* subspecies *multocida, septica* and *gallicida)* are recognized (**Mutters *et. al*., 1985**). *Pasteurella multocida* subspecies *multocida* is the most common cause of disease, but subspecies *septica* and *gallicida* may also cause fowl cholera-like disease to some extent (**Hirsh *et al*., 1990**). *Pasteurella multocida* subspecies *gallicida* is mainly associated with web-footed birds (**Gooderham, 1996; Christensen and Bisgaard, unpublished observations**), but has also been reported in pigs (**Cameron *et al*., 1996**).

**2.2 Morphology:**

*Pasteurella* *multocida* is a gram negative, non-motile, non-spore forming rod shaped bacteria. They arranged singly or in pairs or in cluster (**Carter, 1984**). Majority of the serotypes are found to be capsulated and exhibit bipolarity (**Dwivedi and Siddhi, 1989**).

**2.3 History:**

Several epornitics among fowl occurred in Europe during the latter half of the 18th century. The disease was studied in France by Chabert in 1782 and in 1836 by Mailet, who first used the term fowl cholera. Benjamin in 1851 gave a good description of the disease and demonstrated that it could be spread by cohabitation. *P.multocida* was first found in 1878 in cholera-infected birds. However, it was not isolated until 1880 by Louis Pasteur- the man in whose honour Pasteurella is named (**L Pasteur, The attenuation of causal agent of fowl cholera**).

Huppe in 1886 referred to ‘hemorrhagic septicemia’ and Lignieres in 1990 used the term avian pasteurellosis. In 1879, Toussaint isolated the bacterium and proved it was the sole cause of the disease.

**2.4 Antigenic Nature**:

 The types (or serogroups) of *Pasteurella* *multocida* are identified on the basis of differences in capsular substances (polysaccharides) and are designated as A,B,D,E (**Namioka and Murata, 1961)** and F  **(Rimler and Rhoades, 1987**). Capsular types may be subdivided further into somatic types on the basis of serologic differences in lipopolysaccharides (somatic or O antigens). For many years, passive haemagglutination tests have formed the basis for a serogrouping system based on specific capsule antigens (**Carter, 1955; OIE, 1996**), whereas tube agglutination and gel diffusion precipitin tests have been used to detect somatic antigens (**Heddleston *et al*., 1972; Namioka & Murata, 1961; OIE, 1996**). Sixteen somatic serovars (1-16) are currently recognized (**Rimler and Rhoades, 1989**). All but serotypes 8 and 13 have been isolated from avian hosts (**OIE, 1996**), as have capsular types A, B, D and F (**Rimler & Rhoades, 1989; Rimler & Glisson, 1997**).

However, subspecies *multocida* and serovar A appear to be the most frequently isolated subspecies and serogroup from cases of the most severe form of fowl cholera (**Rhoades and Rimler, 1987; Rimler and Rhoades, 1989**). **Lee *et al., (*1988)**demonstrated that different isolates of the common serovar A: 3, 4 vary greatly in virulence.

**2.5 Pathogenicity:**

Virulence properties of the different subspecies for different avian hosts are unclear. No single factor has been associated with the strain variation in virulence observed (**Lee *et al*., 1988**). The capsule is regarded as a major virulence factor of avian *P*. *multocida* (**Tsuji & Matsumoto, 1989; Harmon *et al.,* 1992**). Inability of virulent strain to produce capsule may yield loss of virulence (**Heddleston *et* *al*., 1964**). But other factors probably influence the outcome of infections.

Other virulence factors suggested include the following:

- endotoxin (**Coy *et al.,* 1997; Ficken & Barnes, 1989; Ficken *et al*.,1991; Lee *et al.*,1992**)

- outer membrane proteins

- iron binding systems (**Zhao *et al*., 1995**)

- heat shock proteins

- neuraminidase production (**Ifeanyi & Bailie, 1992; Lee *et al*., 1994**)

- antibody cleaving enzymes (**Pouedras *et al*., 1992**).

Toxins other than endotoxin may also play a role in the pathogenesis of fowl cholera. *Pasteurella multocida* toxin, which is at least partly responsible for the lesions observed in atrophic rhinitis in pigs, cannot be excluded as a possible virulence factor in some of the lesions observed in avian infections with *P*. *multocida* (**Christensen & Bisgaard, 1997**). Production of RTX toxins (repeats in toxin), which is of major importance in the pathogenesis of some members of the family *Pasteurellaceae* **(Fenwick, 1999**), has not been observed in *P.* *multocida.* One of the few factors which may be of practical value as a virulence marker is the ability of *P*. *multocida* to resist killing by serum components **(Lee *et al*., 1988).** Very little is known about the molecular basis of diseases caused by *P*. *multocida* in avian species (**Christensen & Bisgaard, 1997**) and genetic evidence for the role of virulence factors is lacking, even for some of the factors currently considered to have the most influence over virulence (e.g. the capsule) (**Adler *et al*., 1999**). Factors other than those associated with the bacteria may influence the outcome of *P*. *multocida* infections. Although most species of birds are considered susceptible to infection with *P*. *multocida* (*P*. *multocida* has been isolated from more than 100 different species of birds [**Botzler, 1991**]), different species of birds differ significantly in susceptibility to infection. Among domestic fowl, turkeys are probably the most susceptible species. Web-footed birds also seem highly sensitive to infection, since outbreaks regularly cause massive losses among waterfowl (**Botzler, 1991**), whereas chickens are considered relatively resistant (**Rimler & Glisson, 1997**). Other factors which have been reported to affect the severity and incidence of the disease include environmental factors (e.g. crowding), climate (**Simensen *et al*., 1980**), concurrent disease (**Collins, 1977**), nutritional stress (**Eveleth *et al*., 1949**) and age of the host (**Hungerford, 1968**). Age markedly influences the outcome of infection, at least in chickens, where birds less than sixteen weeks old are relatively resistant. Under natural conditions, mortality may range from only a few percent to close to 100%, depending on the factors mentioned above (**Rimler & Glisson, 1997**).

 Turkeys are much more susceptible to infection with *P. multocida* than chickens and mature chickens are more susceptible than young chickens (**Heddleston, 1962**).

**Rahman *et al*., (2004)** reported that fowl cholera was recorded in chickens more than two weeks of age with significantly highest occurrence in adult chickens; the prevalence of fowl cholera was also found significantly highest during summer (49.12%) in comparison to rainy (26.32%) and winter (24.56%) seasons and the incubation period is 96 hours and clinical signs appear after 4th day of inoculation at 52 day old broiler chicken and caused 100% morbidity.

**Islam *et al.,* (2008)** confirmed the virulence and pathogenicity of chicken isolate of P. multocida in ducks.

**2.6 Epidemiology:**

Although fowl cholera probably occurs world-wide (**Rimler & Glisson, 1997**) and has been studied extensively for many years, the epidemiology of the disease remains controversial, and many aspects are not yet fully understood. Basic knowledge, such as the route of introduction of fowl cholera into a flock, is still lacking. Due to genotypic variation within serotypes, serotyping in many cases does not provide sufficient detailed information to determine the epidemiology of infections (**Christensen *et al*., 1998; Kim & Nagaraja, 1990; Wilson *et al.,* 1993**). Within the last ten years, DNA (deoxyribonucleic acid) fingerprinting in the form of restriction endonuclease analysis and ribotyping has been applied to avian strains of *P*. *multocida* of different origins, including strains obtained from wild birds (**Christensen *et al*., 1999).** The restriction endonucleases Hpall and Hhal are reported to be among the most suitable for epidemiological studies (**Christensen *et al*., 1998**; **Wilson *et al*., 1995**).

*P.* *multocida* is a fairly delicate organism which is easily inactivated by common disinfectants, sunlight, drying or heat, and experiments suggest that *P*. *multocida* will survive for a maximum of thirty days in the environment (e.g. water or soil) (**Backstrand & Botzler, 1986; Rimler & Glisson, 1997**). Consequently, contaminated environments are not thought to serve as reservoirs for periods of more than thirty days, although as yet unknown factors could have a protective role. As the habitat of *P*. *multocida* is broad, including mucosal surfaces of mammals, birds and humans, many sources could act as a potential reservoir (**Bisgaard, 1993**). An exchange of *P.* *multocida* ssp. *multocida* between wild birds and domestic poultry is reported to be possible, and wild birds are capable of spreading the disease to new areas (**Christensen *et al*., 1999; Snipes *et al.,* 1990**). A recent study demonstrated that more than 80% of the diagnosed cases of *P*. *multocida* infections in poultry in Denmark during the years 1995 to 1997 included poultry which had been in contact with wild fauna (**Christensen *et al*., 1999**). However, dogs, cats and pigs, in particular, may act as reservoirs for strains of *P.* *multocida* which are virulent for poultry (**Rimler & Glisson, 1997; Van Sambeek *et al*., 1995**). Carrier birds are generally believed to play a major role in spreading the disease (**Carpenter *et al.,* 1989; Rimler & Glisson, 1997**). An investigation by **Muhairwa *et al****.* has indicated that a high carrier rate of *P*. *multocida* ssp. *multocida* and *septica* may exist in apparently healthy poultry flocks (including chickens and ducks). Surprisingly, many birds carried *P*. *multocida* on the cloacal mucosae. The importance of this finding in explaining the spread of the infection is unclear, as excretions from the mouth, nose and conjunctiva of diseased birds are generally believed to be the primary source of contamination of the environment; transmission by aerosol has been reported to be less important (**Bierer & Derieux, 2003**). Other potential sources of infection are carcasses of birds which have died of the infection and equipment or insects which have been in contact with infected birds. Transmission of *P*. *multocida* through the egg is not believed to represent a risk (**Rimler & Glisson, 1997**), although contamination of eggshells could theoretically occur during passage through the cloaca. However, given the delicate nature of the micro-organism, contamination of this type is likely to be insignificant.A study of more than 2000 fresh and embryonated eggs from chickens infected with chronic fowl cholera yielded no evidence that *P*.*multocida* was transmitted through the egg (**Report of the Chief of BAI, USDA, 1951**).Carrier birds harbor bacteria in their nasal plates and exertion takes place through month. Poultry droppings generally do not contain viable *P.multocida* (**National Wildlife Health Centre, U.S.A., 1999**).

**2.7 Disease:**

The site of infection for *P. multocida* is generally believed to be the respiratory tract (**Rimler & Glisson, 1997; Maheswaran *et al*., 1973**). However, inoculation through oculo-nasal-oral routes may also generate typical lung lesions and a progressive bacteraemia (**Prantner *et al*., 1990**), indicating that other mucosal membranes may serve as portals of entry. The ability of *P*. *multocida* to survive passage of the gastro-intestinal tract appears to be limited (**Rimler & Glisson, 1997**), but the presence of *P*. *multocida* on the cloacal surface of carrier birds indicates that some organisms may survive passage (**Muhairwa *et al*., 2000**). The observation that some strains of *P*. *multocida* can be virulent and immunogenic following oral administration also suggests that intestinal invasion or interaction with the intestinal mucosae occurs to some degree (**Lee *et al*., 1988; Frost *et al.,* 1999**). Localisation of *P*. *multocida* in the bursa may occur following a bacteraemia, since *P*. *multocida* has been detected in the bursa of intratracheally infected chickens (**Christensen and Bisgaard, unpublished data**).

*Pasteurella multocida* may also enter the tissues through cutaneous lesions and result in septicaemia (**Rimler & Rhoades, 1989**) or localized cutaneous lesions (**Frame *et al*., 1994; Glass & Panigrahy, 1990**). Following an upper respiratory tract infection, *P*. *multocida* may subsequently spread to the lungs and multiply before entering the bloodstream (**Matsumuto et al., 1991**). Once in the bloodstream, *P*. *multocida* either multiply rapidly (**Snipes *et al*., 1987**) or localise in the liver and spleen where initial multiplication occurs before a massive bacteraemia (**Tsuji & Matsumoto, 1989**). Death is presumed to be due to the effects of endotoxin (**Rhoades, 1964; Heddleston *et al.,* 1964; Collins, 1977**), as signs of acute fowl cholera have been reproduced by injection of endotoxin from P. *multocida* (**Heddleston *et al.,* 1975; Rhoades & Rimler, 1987**).

**2.7.1 Clinical findings:**

 Few signs may be observed in per acute and acute infections (often referred to as cholera). In these cases, death is often the only sign of disease in the flock. In more protracted cases, mucous discharges from the mouth, nose and ears, cyanosis, general depression, ruffled feathers and greenish diarrhea may be observed. In chronic infections, signs are principally due to localized infections of leg or wing joints, comb, wattles and subcutaneous tissue of the head (**Gustafson *et* *al.*, 1998; Rimler and Glisson, 1997**), oviduct (**Bisgaard, 1995**) and the respiratory tract (**Rimler and Glisson, 1997**). Severe forms of dermal necrosis in turkeys have also been reported (**Frame *et al.,* 1994; Glass & Panigrahy, 1990**).

**2.7.2 Lesions:**

According to **Rimler and Glisson (1997),** in the case of peracute or acute forms of the disease, the post-mortem findings are dominated by general septicaemic lesions including vascular disturbances, as reflected by general passive hyperaemia and congestion throughout the carcass. Petechial and ecchymotic haemorrhages are present in the abdominal and coronary fat, and haemorrhages may be observed in the intestinal mucosae and on subserosal surfaces in the thoracic and abdominal cavities. The liver and spleen are often swollen and may contain multiple small focal areas of coagulative necrosis or the organs may undergo more generalised necrosis. In the most acute forms of infection, the lung lesions are dominated by haemorrhages, but this is soon followed by necrosis and fibrinous pleuro-pneumonia where affected areas are clearly marked from unaffected tissue. A unilateral or bilateral productive inflammation of pleura and lungs with extensive exudation of fibrin is common.

Histologically, the lesions are mainly associated with heterophilic infiltrations (**Rimler and Rhoades, 1989; Rimler and Glisson, 1997**). In chronicinfections, suppurative lesions may be widely distributed, often involving the respiratory tract, the conjunctiva and adjacent tissues of the head (**Rimler and Glisson, 1997**). Caseous arthritis and productive inflammation of the peritoneal cavity and the oviduct are common in chronic infections. A fibrino-necrotic dermatitis including caudal parts of dorsum, the abdomen and breast, and involving cutis, subcutis and the underlying muscle, has been observed in turkeys and broilers (**Frame *et al*., 1994; Glass & Panigrahy, 1990**).

**2.8 Diagnostic methods:**

The history of the disease, clinical signs and gross lesions may be helpful in diagnosis, but are insufficient to allow a definite diagnosis of the disease. The final diagnosis depends on isolation of the organism. Primary isolation is usually accomplished using media such as blood agar, dextrose starch agar. Isolation may be improved by the addition of 5% heat inactivated serum (**OIE, 1996**). *P. multocida* can be readily isolated from viscera of birds dying from per acute/acute fowl cholera and often from suppurative lesions of chronic cases. In cases of acute fowl cholera, bipolar organisms can be demonstrated in liver imprints using Wright's or Giemsa stain (**OIE, 1996; Rimler and Glisson, 1997).**

Colony appears after incubation on dextrose starch agar for 24 hours at 370 C in air or in atmosphere of 6-8% CO2.They are usually of moderate size, 2-2.5 mm in diameter, convex, circular, smooth and iridescent (**Brogden *et al*., 1977**). Immunofluorescent microscopy has been used to identify *P*. *multocida* in tissue and exudates (**Rimler & Glisson, 1997**). More recently, the polymerase chain reaction technique has been used with success to detect carrier animals within turkey flocks (**Kasten *et al*., 1997**). For surveillance purposes and to investigate for carrier animals, the most sensitive method still appears to be mouse inoculation (**Kasten *et al*., 1997; Muhairwa *et al.,* 2000**). Isolation attempts on selective media or blood agar may represent an alternative (**Moore *et al*., 1994**), but this method appears to be less sensitive than mouse inoculation (**Muhairwa *et al*., 2000**). Following isolation, identification is based on the results of biochemical tests. The fermentation of dextrose, sucrose and mannitol without gas production and the inability to ferment lactose and maltose were the criteria used to identify the cultures of *P. multocida* of avian origin. The key characteristic reaction used to identify the cultures is the indole positive reaction (**Dorsey, 1963; Rhodes and Rimler, 1991**). **Donahue and Olson (1971)** showed production of indole in SIM media without H2S.

**Table 1: Characteristics used for identification of subspecies of *Pasteurella multocida***

|  |  |
| --- | --- |
| Characteristics | Pasteurella multocida |
| ssp. multocida | ssp. septica | ssp. gallicida |
| L (+)Arabinose | D | - | + |
| D(-) Arabinose | D | D | - |
| Dulcitol | - | - | + |
| D (-) Sorbitol | + | - | + |
| L (-) Fucose | D | D | - |
| Trehalose | D | + | - |
| α glucosidase | + | + | - |

+ : > 90% of strains positive within one to two days

- : >90% of strains negative

D: different

Serological tests for the presence of specific antibodies are not used for diagnosis of fowl cholera, but have been used in order to test immunity in vaccinated poultry (**OIE, 1996**).

**AKM Rakibul Hasan et al., (2010)** found 4.25% and 12.5% fowl cholera in broiler and layer respectively based on clinical signs and PM examination; but at laboratory diagnosis they found no fowl cholera in broiler and 9.09% fowl cholera in layer.

**2.9 Public health implications:**

Disease in humans caused by *P.* *multocida* is not uncommon, and *P*. *multocida* may be considered a zoonotic organism (**Bisgaard *et al*., 1994**). This is substantiated by the observation that the disease apparently occurs predominantly among the farming population (**Bisgaard *et al*., 1994**). No reports exist of direct transmission from poultry to man or vice versa, but the possibility for such infections cannot be excluded. The organism is a common cause of infection following animal bites or scratches which are mostly caused by dogs or cats (including large cats). Bite wound infections caused by pigs have also been reported (**Frederiksen, 1993**). A severe cellulitis may develop which may progress to osteomyelitis and subsequently to septicaemia (**Francis *et al*., 1975**). *P. multocida* may also be involved in respiratory tract infections, either as a primary or secondary infectious agent (**Weber *et al*., 1984**). In patients with dysfunction of the liver in particular (**Weber *et al*., 1984**), *P*. *multocida* is known to cause bacteraemia which may localise in joints, respiratory tract or progress and cause sepsis. In addition to these principal types of infections, *P. multocida* has been isolated from a variety of infections, including peritonitis, puerperal sepsis, neonatal sepsis, brain abscesses and urinary tract infections (**Bisgaard *et al*., 1994**).

**2.10 Treatment:**

Drugs like sulphonamides and antibiotics have been used for treatment of fowl cholera. **Donahue and Olson (1972)** used kanamycin and neomycin. Trimethoprim and sulphamethoxasole combination have been used with success.

Ampicilin, tetracycline, doxicycline in the usual dose rate can be used. Tetracycline can be used parenterally in case of outbreak. Penicillin can be used parenterally against sulpha resistant flock.

**Nasrin *et al*., (2007) found** that ampicillin and chloramphenicol are effective against *Pasteurella spp.*

**2.11 Methods of prevention & control:**

Control of fowl cholera throughout the world depends principally, on vaccination. Many live and inactivated (bacterins) fowl cholera vaccines have been developed and tested in attempts to control the disease (**Rimler & Glisson, 1997**). As modified live vaccine strains can revert to their pathogenic phenotypes and tend to cause disease in immunocompromised birds, most commercial vaccines are of the bacterin type. The vaccines normally contain *P. multocida* of serotypes A:l, A:3 and A:4 which has been grown in vitro, emulsified in an oil adjuvant or aluminium hydroxide (**Homchampa, 1995**). Bacterins are inexpensive to produce and provide some degree of protection, consequently limiting the incidence and severity of clinical disease (**Scott *et al*., 1999**). The principal disadvantages of the bacterins are that these vaccines have to be injected, often resulting in tissue reactions (**Davis, 1987**), and only induce immunity to homologous serotypes (**Rebers & Heddleston, 1977**). As a result, the development of safe live vaccines is highly desirable to allow the use of a less laborious route of administration and to obtain cross-immunity.

An oil adjuvant killed vaccine is produced by Livestock Research Institute (LRI) which is given @ 0.5ml/bird at the age of 2.5 months, s/c. Then boostering at every after 6 months.

**Sultana *et al*., (2013)** compared the efficacy of both BAU-FCV and LRI-FCV in Zending duck and stated that both vaccines are safe and effective.

**Samina et al., (2013**) demonstrated that experimental oil adjuvant fowl cholera vaccine with 0.5ml dose produce higher immune response against challenge infection and found to be safe.

**Parvin *et al*., (2011)** reported that breed variation has no significant effect on immune response to fowl cholera vaccine.

**Iqbal *et al****.,* **(2010**) reported that formalin-inactivated alum-precipitated fowl cholera vaccine (FCV) is effective for immunization of coturnix quail.

**Rahman *et al*., (2004)** showed the fact that intramuscular route followed by subcutaneous inoculation could be done for immunization against fowl cholera in chickens.

Avirulent mutant vaccine have been found to be effective (**Maheswaran *et al*., 1973**).

**Dougherty (1953)** compared immunizing properties of chemically killed autogenous broth culture vaccine and a chicken embryo vaccine. He also found that the egg embryo vaccine gave a greater degree of protection than chemically killed broth culture vaccine.

**Chapter-III**

**Materials and Methods**

**3.1 Study area & duration**: The study was conducted at Chittagong during my internship placement at CVASU lab rotation from 7 July to 31 July, 2013. Laboratory works were done at microbiology lab, CVASU.

**3.2 Study Population**: 30 dead layer birds were collected. Some of them were collected from some layer farms and some were collected from patients came to Professor Dr. Saifuddin, Head of the Dept., Dept. of Pharmacology, Biochemistry & Physiology, Professor Dr. Masuduzzaman, Dept. of Pathology & Parasitology for treatment purpose. By interviewing the farmers relevant data of birds were collected.

**3.3 Case definition**: Fowl cholera is an important bacterial disease which is clinically characterized by high fever, anorexia, ruffled feather, mucous discharge from the mouth, greenish diarrhea and increased respiratory rate. Cyanosis often occurs prior to death and is most evident in head, comb and wattles.

Postmortem lesions are associated with vascular disturbances. General hyperemia usually occurs, is most evident in veins of the abdominal viscera, and may be quiet pronounced in small vessels of duodenal mucosa. Petechial and ecchymotic hemorrhages are frequently found and may be widely distributed. Livers of acutely affected birds may be swollen and contain multiple focal areas of coagulative necrosis and heterophilic infiltration. Sub-epicardial and sub-serosal hemorrhages are common, as are hemorrhages in the lungs, abdominal fat and intestinal mucosa. Increased amount of pericardial and peritoneal fluid are frequently occurred. (According to **BW Calnek**).

**3.4 Methods of study:** A total number of 30 postmortem cases of layer birds were observed during the study period. Fowl cholera was diagnosed by clinical history, clinical findings and postmortem lesions. For every case, liver was collected because this is the common site of occurring lesions as well as harboring organisms of fowl cholera.

**3.5 Microbiological examination of collected sample**: Smears were prepared from liver impression. Four slides were made for one sample. Among them two were stained with Gram’s staining and another two were stained with Giemsa stain. In case of Gram’s staining, gram negative, coccobacilli, capsulated bacteria were seen in positive cases.

For Giemsa stain, the slides were fixed in methanol for 3-5 minutes, dried in air and stained with Giemsa stain. Giemsa stock solution was made by mixing Giemsa powder 0.3 gm, glycerin 25.0 ml and methanol 25.0 ml. Then the solution was filtered. To make the working solution, 1volume stock solution was diluted with 10 volume of distilled water. After staining, the slide was examined under microscope. Under compound microscope, at 100x, bipolar bacteria was seen in positive case of fowl cholera.

**3.6 Prevalence calculation:** Prevalence was calculated with the help of MS Excel 2007. The apparent prevalence of fowl cholera is calculated by the number of cases of fowl cholera (diagnosed by postmortem lesions and lab test) as the numerator and the total number of dead/sick chicken presented as the denominator. Again the true prevalence is calculated by the number of fowl cholera positive as the numerator and the total population as the denominator.

**Evidence of postmortem and laboratory examination**

 

Fig: Postmortem of layer birds Fig: Multiple necrotic foci in enlarged liver

 

Fig: Taking impression smear from liver Fig: Giemsa staining

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**CHAPTER-IV**

**RESULT AND DISCUSSION**

Table 2: Population data

|  |  |  |
| --- | --- | --- |
| Sl. No. | Name of Farm | No. of total birds |
| 1 | F1 | 1000 |
| 2 | F2 | 1200 |
| 3 | F3 | 1500 |
| 4 | F4 | 1000 |
| 5 | F5 | 2000 |
| 6 | F6 | 1200 |
| 7 | F7 | 1500 |
| 8 | F8 | 1000 |
|  | Total | 10400 |

Table 3: Postmortem and laboratory examination

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| SL. NO. | Name of Farm | No. of dead/sick birds | Fowl cholera positive | Fowl cholera negative |
| 1 | F1 | 4 | - | 4 |
| 2 | F2 | 3 | - | 3 |
| 3 | F3 | 4 | - | 4 |
| 4 | F4 | 3 | - | 3 |
| 5 | F5 | 5 | 3 | 2 |
| 6 | F6 | 3 | - | 3 |
| 7 | F7 | 4 | - | 4 |
| 8 | F8 | 4 | - | 4 |
|  | Total | 30 | 3 | 27 |

**Table 4: Prevalence of fowl cholera in postmortem of layer birds**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sl. No. | Basis | Fowl cholera Positive | Total number | Prevalence of fowl cholera affected farm | Apparent prevalence | True prevalence |
| 1 | Farm | 1 | 8 | 12.5% |  |  |
| 2 | Sample | 3 | 30 |  | 10% |  |
| 3 | Total population | 3 | 10400 |  |  | 0.029% |

Data analysis by MS Excel 2007.

In postmortem examination, must striking point such as white necrotic foci on enlarged liver, multiple petechial hemorrhages throughout the viscera were noticed. The impression smear from liver revealed bipolar bacteria under microscope.

The apparent prevalence of fowl cholera in target population is 10% based on postmortem examination and laboratory test. The prevalence of fowl cholera affected farm is 12.5%. The true prevalence of fowl cholera is 0.029%. The result may be due to improper hygiene e.g.; absence of footbath, poor ventilation etc. There was also age variation; most of the reference population was more than 20 weeks of age. Besides this, the result is not very significant due to very small sample size.

**Uddin *et al*., (2010)** reported the prevalence of fowl cholera at Norshingdi is 0.24%. **Islam *et al.,* (2003)** reported that the prevalence of fowl cholera in Sylhet region of Bangladesh is 0.44%. **Giasuddin *et al.,* (2002**) reported that prevalence of fowl cholera is 3.08% in Bangladesh.

**Rashid *et al*., (2013)** reported that 3.23% mortality is attributed by fowl cholera in commercial layer chickens of Bangladesh.

**Dutta *et al.,* (2013)** reported that 32.29% of indigenous chickens are died from fowl cholera in some selected areas of Rajshahi, Bangladesh.

**MZ Uddin (2011)** reported that fowl cholera is the cause of 0.46% mortality in ISA-Brown and Hy-Line strain of layer chicken reared in cage in Bangladesh.

**Biswas PK *et al*., (2007)** showed the mortality rate by fowl cholera is 6.76% in semi-scavenging chickens in Bangladesh.

**Rahman *et al*., (2004)** reported that fowl cholera was recorded in chickens more than two weeks of age with significantly highest occurrence in adult chickens; the prevalence of fowl cholera was also found significantly highest during summer (49.12%) in comparison to rainy (26.32%) and winter (24.56%) seasons and the incubation period is 96 hours and clinical signs appear after 4th day of inoculation at 52 day old broiler chicken and caused 100% morbidity.

**Haider *et al.,* (2004)** found that the percentage of fowl cholera microorganism is 3.33% in the intestines of Sonali chicken.

**Rahman *et al*., (2003)** reported that fowl cholera causes 6.52% mortality in commercial chickens in Bangladesh.

**Limitations:**

* The study period was very short because most of the time of our internship we were at different regions of our country except at the time of lab rotation.
* There are few number of layer farm at metropolitan area; most of the layer farms are located outside the city. Due to time shortage and inability to go to all the layer farms there is reduction of number of sample and cases.
* Inability to perform serological tests e.g.; FAT, ELISA etc.

**CHAPTER-V**

**CONCLUSION**

General septicemic changes (hyperemia, congestion, petechial hemorrhages) in viscera, multiple necrotic foci in enlarged liver were the predominant changes observed in fowl cholera affected layer birds. They might be the major changes in fowl cholera affected chickens in Bangladesh and therefore might be referred to the diagnosis of fowl cholera in the country in general. Though postmortem and clinical manifestations help to diagnose, by Giemsa staining we can diagnose confirmly. The apparent prevalence of fowl cholera is 10% and fowl cholera affected farm is 12.5%. But the true prevalence of fowl cholera is 0.029% in the respective study area. Laying chickens are more susceptible to fowl cholera than younger ones. The study may be extended in wider scale to other different areas of the country to verify and compare the results and to take necessary steps against *P*.*multocida* infections. However, poor management and lack of awareness of farmers also enhances the infection of *P. multocida*. So, preventive measures should be taken and birds should be vaccinated regularly.

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**ANNEX**

1. Basic information:
2. Name of the farm :
3. Name of the owner:
4. Address of the farm:
5. Farm size :
6. Housing pattern and management:
7. Housing system : Intensive / Semi-intensive
8. Floor type : Litter /concrete / slat / cage
9. Ventilation status of house : poor / moderate / good / nothing
10. Drainage status : poor / moderate / good / nothing
11. Frequency of cleaning the house : once a week/ twice per week / daily / other schedule
12. Biosecurity : Visitor access- restricted / not restricted
13. Foot bath : present / absent
14. Trees around the farm : present / absent
15. Feeding system : scavenging / supplement / both
16. Preventive measures :
17. Regular practice of vaccination : Yes /No
18. Types of vaccine used :