2. REVIEW OF LETERATURE

2.1. Incidence of *P. multocida* infection in the world:

Sander and Glisson (1989) isolated *P. multocida* from six broiler flocks in Georgia at age from 20 to 46 days during Summer 1988. Increased mortality and lameness were the clinical signs that presented in all affected flocks. Bacterial isolation and agar gel precipitation for somatic antigen serotyping revealed that three of the cases were caused by serotype 1 and 3 , two by serotype 3 and 4 and one by serotype 3.

Waltman and Horne (1993) diagnosed 176 cases of FC from Georgia in 1989-1991 with high incidence during March and April. Serotypes distribution of isolates showed that serotype 3 and 4 were predominant (40%) followed by serotype 3 (22%); serotype 1 (18%); untytpable (15%); serotype 5 (3%) and serotype 4 (2%) .

Rajini et al., (1995) isolated and identified *P. multocida* from chickens suspected for FC in Andhra Pradesh, India. A total of 30 isolates were recovered and were confirmed on the basis of morphological, cultural, biochemical and pathological characters.

Poernomo and Sarosa (1996) isolated *P. multocida* from five, 32 day old broiler chickens in the late of 1992 from a farm located in Bogor area, Indonesia. Serological test of the isolates revealed presence of serotype A.

Christensen et al., (1998) characterized phenotypically and genotypically *P. multocida* isolates from back-yard poultry including chickens, pheasant, turkeys and ducks and from two outbreaks from wild birds in Denmark in 1996. The results showed that all isolates were identical, indicating a possible exchange of *P. multocida* ssp. multocida between population of wild birds and back-yard poultry.

Jonas et al., (2001) reported on 20 field outbreaks of FC in Indonesia during 1998-99 in chickens. Nine isolates of *P. multocida* were recovered from these field outbreaks. Of 9 Indonesian isolates, three were capsular type A, one isolate was of type B and one of type F. For 3 isolates, the capsular serogroup could not be identified.

Parveen et al., (2003) isolated *P. multocida* from a breeder chicken flock at age 245 days in Lahore district, Pakistan in January 2003.

Pedersen et al., (2003) recorded an outbreak of FC among wild birds in Denmark in 2001.

Zhang et al., (2004) described 2 outbreaks of FC in a multiage free range layer farm in Australia. The outbreaks occurred in 1994 and 2002. A total of 22 strains of *P. multocida* were available for study, 11 from the 1994 outbreak and 11 from the 2002 outbreak.

Shivachandra et al., (2005) investigated 2 FC outbreaks that occurred within one month (first outbreak in the last week of September and second outbreak in the third week of October 2002) separately in two different flocks in a single private chicken farm, at Maharashtra, India. Those outbreaks were recorded among layer flocks of the 15 to 16-week-old age. A total of 12 and 18 strains of *P. multocida* obtained from two outbreaks.

Jabbari et al., (2006) applied molecular methods for detection and capsular typing of 39 avian *P. multocida* isolates from Iran. The PCR amplified a fragment of 1044 base pair (bp) from all of tested isolates. It was found that all avian *P. multocida* isolates belonged to capsular type A.

Masdooq et al., (2008) isolated 32 isolates (6.5%) of *P. multocida* out of 500 lung samples with pneumonia from chickens slaughtered at abattoir in Jos South, Nigeria.

Mbuthia et al., (2008) succeeded in isolation of *P. multocida* from 25.9% of apparently healthy ducks and from 6.2 % of free range chicken poultry farms and at slaughter slabs at market.

Hasan et al., (2010) stated that in Bangladesh, the prevalence of FC in layers was 12.5% and in broilers was 4.25%.

Kim et al., (2011) described an outbreak of FC in 13-week-old broiler breeder chickens in April 2006 in Korea. Eleven strains of *P. multocida* were isolated and identified as capsular serogroup A.

Balakrishnan and Parimal (2012) isolated 8 *P. multocida* isolates from the heart blood, spleen, liver and lung of turkeys and chickens and identified them on the basis of biochemical characteristics, pathogenicity studies in mice and PCR .

Chrzastek et al., (2012) reported 42 clinical isolates of *P. multocida* from various avian hosts (geese, ducks, turkeys, and laying hens) in Poland from 2001 to 2011 where all isolates were identified as *P. multocida* serotype A.

Levy et al., (2013) reported that 25-35% mortalities in chickens in Bangladesh were caused by FC outbreaks.

Pillai et al., (2013) isolated *P. multocida* from poultry using standard bacteriological procedures for a period of 2 years. Ten isolates were obtained from 155 samples. All isolates have been serotyped as A:1.

Rigobelo et al., (2013) studied the incidence of *P. multocida* in Brazil in 8 groups of 90 birds each. Groups I to IV were from chickens (I being > 6 weeks of age with a history of respiratory illness, II > 6 weeks of age and free of respiratory illness, III < 6 weeks of age with respiratory illness and IV being < 6 weeks of age and with no respiratory illness). Groups V to VIII had the matching characteristics of Groups I to V but consisted of Japanese Quails. The *P. multocida* isolation rate was as follows, group I 56/90 (62.3%), group II 18/90

(20.0%), group III 12/90 (13.3%), group IV 3/90 (3.33%), group V 8/90 (8.88%), group VI 2/90 (2.22%) group VII 2/90 (2.22%) and group VIII 1/90 (1.11%).

Bhimani et al., (2014) isolated 22 isolates of *P. multocida* out of 168 samples (bone marrow, lung, liver, and blood) from 42 dead bird suspected to be died from FC.

Castillo et al., (2014) isolated 13 strains of *P. multocida* from secretions and internal organs of broilers and layers from various poultry farms in the coast and the tropics of Peru.

Furian et al., (2014) succeeded in isolation of 54 strains of *P. multocida* from FC cases in Southern Brazil where 41 strains (75.93%) were classified into serogroup A and only 11 isolates (20.37%) were unidentifiable.

Zahoor et al., (2014) reported that 6 *P. multocida* isolates were recovered from 43 liver samples collected from poultry flocks in and around Faisalabad, Pakistan.

Panna et al., (2015) isolated *P. multocida* from 11.42 % (n=4/ 35) samples in Gazipur and Pabna districts, Bangladesh.

Akhtar et al., (2016) isolated *P. multocida* from 2 out of 5 suspected dead birds that collected from breeders farm located at Gazipurdistrict, Bangladesh.

Mehmood et al., (2016) examined 5 diseased birds showing respiratory syndromes from each of five commercial layer chicken flocks in Karachi, Lahore, Vehari and Toba Tek Singh, Pakistan. Clinical signs, postmortem changes, cultural and microscopic characteristics, biochemical and molecular characterizations confirmed that the causative agent of the disease was *P. multocida* serotype A.

2.2. Incidence of *P. multocida* infection in Egypt:

Farag (1977) studied the incidence of pasteurellosis in ducks. Fifteen *P. multocida* strains were isolated from living and dead ducks.

Hussin (1988) isolated 31 *P. multocida* out of 359 examined cases from layers in Sharkia province. *P. multocida* was isolated from different organs as following: 35.48%, 29.03%, 16.13%, 9.67%, 6.45% and 3.22% from heart blood, liver, ovary, nasal cleft, bone marrow and lung; respectively.

Abd El-Dayem (1990) recorded that a total of 500 samples from infected and apparently healthy ducks, turkeys and chickens were collected from Kaloubia province where 45 (9%) isolates of *P. multocida*were recovered. Ducks showed the highest frequency of isolation where the incidence was 12% followed by turkeys (10%) and chickens (3.3%). The results obtained by determining both the capsular and somatic antigens, showed that, the 45 *P. multocida* strains isolated from ducks, turkeys and chickens were classified into: 5:A (23) isolates, 8:A (10) isolates, 9:A (8) isolates and 2:D (4) isolates.

Nagi et al., (1990) studied several outbreaks of FC in breeder poultry farms in Egypt. The causative organism was shown to be *P. multocida* serotypes 5:A and 8:A.

Ibrahim (1991) isolated 26 serotypes of *P. multocida* from ducks over 10 weeks of age. Serological identification revealed that the isolates were serotype 3, 4 and 5.

Hassan et al., (2001) isolated *P. multocida* type D and A: 3,4 from laying chicken flocks.

Hanan (2004) serologically identified 4 ducks strains of *P. multocida* and revealed that they belonged to serotypes A:1, A:3, A:4 and D:11.

EL-Shamy (2008) isolated 14 strains of *P. multocida* from 128 diseased and dead ducks represented 21 farms. The percentage of *P. multocida* isolation was 13.81. The highest percentage of isolation of the organism was in El-Sharkia governorate (8.57%) followed by El-Garbia governorate (2.94%), then EI-Behera governorate (2.3%).

Mohamed et al., (2012) reported that out of 275 examined samples from backyard chickens from different regions of Upper Egypt, 21 (7.6%) isolates of *P. multocida* were recovered and confirmed using phenotypic characterization. Somatic serotyping of the 21 isolates resulted in 12 isolates being classed as serotype A:1 (57.14%), 4 as serotype A:3 (19.05%) and 5 could not be typed (23.8%). Capsular typing using multiplex PCR, demonstrated that 18 strains were capsular type A (85.7%), and 3 were type D (14.3%).

Mona (2015) reported that out of 200 examined samples from different governorate of Egypt (135 chickens, 56 ducks and 9 turkeys), 8 isolates (6.25%) of *P. multocida* were recovered from layer chickens. No *P. multocida* could be isolated from ducks, turkeys, and broiler chickens. Molecular serotyping revealed that all 8 isolates were *P. multocida* type A.

Shimaa (2016) isolated 14 isolates of *P. multocida* from commercial broiler chicken farms in the governorate of El-Gharbia at the period of 2011-2014 with rate of incidence 7%. Serological identification revealed that the isolates were serotypes A:1 and A:4.

Samar (2017) reported that a total of 81 freshly dead birds (51 chickens and 30 ducks) were collected from sixteen chickens and six ducks flocks during the period from July 2013 to November 2016 from different farms and house breeding in Dakahlia Governorate. *P. multocida* were isolated from 22 chickens and 16 ducks based on case history, postmortem lesions, bacteriological isolation, PCR, and capsular typing. Serotype A was widely distributed in all examined organs of infected ducks than in chickens.

Ayman (2017) investigated presence of *Pasteurella* infections in water fowl (ducks and geese) in El-Minia governorate. Out of 230 examined cases (180 from ducks and 50 from gees), 15 strains were isolated. Thirteen isolates were recovered from ducks (7.22%) and two isolates were recovered from geese (4%), nine isolates being classified as serotype A:1 (60%), 3 as serotype A:3 (20%), 2 as serotype A:4 (13.3%) and one untypable strain (6.6%).

2.3. Susceptibility to *P. multocida*:

Ghazikhanian et al.,(1982) stated that FC is highly contagious disease affecting many classes of wild and domesticated birds including turkeys, chickens, ducks, geese and pheasants. The turkey was highly susceptible to this disease.

Sander and Glisson (1989) mentioned that FC is more common in layers than in broilers due to age factors. The disease is more common in broiler breeder than broiler.

Rhodes and Rimler (1990) reported that all avian species are susceptible to FC, and turkeys may be the most severely affected species.

Botzler (1991) reported that FC has been recorded in a wide range of avian species, suggesting that all types of birds are susceptible with variation in host susceptibility.

Petersen et al., (2001) reported that *P. multocida* subsp. multocida isolated from outbreaks of FC in wild birds in Denmark was highly virulent for turkeys, partridges and pheasants, while chickens were more resistant. The present finding underlined the importance of wild birds as reservoir for *P. multocida*.

Mbuthia et al., (2008) reported on the occurrence of *P. multocida* among healthy appearing family poultry, and demonstrated that age susceptibility was the highest in 12 week-old family chickens and 8 week old family ducks when challenged with a low virulent strain of *P. multocida*. It had further demonstrated that cross-transmission of FC may happen between family ducks and chickens, and vice versa.

Wang et al., (2009) mentioned that turkeys are more susceptible than chickens to infection with *P. multocida*, and the aged birds are more susceptible than younger.

2.4. Clinical signs and post-mortem lesions of *P. multocida*:

2.4.1. Clinical signs:

Curtis et al., (1980) reported that the signs of FC appeared as depression, torticollis, lameness, conjunctivitis and dyspnea. Swelling of wattle, sinuses, footpads and sterna bursa could occur.

Rhoades and Rimler (1990) mentioned that the clinical signs of FC were anorexia, fever, ruffled feather, mucus discharge from mouth, increased respiratory rate and diarrhea.

Gustafson et al., (1998) noticed the clinical signs of seven, 18 wk- old pullets infected with FC from a commercial layer flock as increased mortality associated with neurologic and respiratory symptoms. Clinical signs included depression, torticollis, swollen eyelids, conjunctivitis and sinusitis.

Chin and Goshgarian (2001) showed that the clinical signs in *P. multocida* infected 8-wk-old ring-necked pheasants were severe sinusitis. The birds showed severe unilateral or bilateral distention of the sinuses by mucoid to caseous exudate. Mortality and morbidity rates were low.

Glisson et al., (2003) mentioned that FC usually appeared as a septicemic disease associated with high morbidity and mortality.

Woo and Kim (2006) recorded symptoms including; orofacial edema, swollen and edematous comb and wattles, and severe respiratory disorders in FC outbreaksof domestic poultry in two broiler breeder farms.

Glisson et al., (2008) recorded that the signs observed due to acute form of FC were fever, anorexia, ruffled feathers, mucous discharge from the mouth, nose and ears, cyanosis of comb and wattles, increased respiratory rate and diarrhea.

Chrzastek et al., (2012) reported that acute form of FC induced high mortality rates, whereas chronic infection was associated with localized infections of sinuses and joints, resulted in persistence of the bacteria within the flock.

Mehmood et al., (2016) recorded the clinical signs of FC in commercial layer flocks with respiratory signs. The mortality rate was 10% and egg production loss was 30%. The birds were depressed, anorexic with ruffled feathers and showed mucous discharge from mouth and nares, inflamed joints and cyanotic comb and wattles.

2.4.2. Post-mortem lesions:

Hunter and Wobeser (1980) observed that vascular damage, general hyperemia and focal necrosis in liver, spleen and other organs were usually occur in acute FC. Localized lesions in a variety of organs including brain, joints, air sacs, lung and eyes occur in chronic infection.

Sakurai et al., (1986) noticed gross lesions as multiple necrotic foci in liver and spleen, and hemorrhages in small intestine in twenty-eight (19%) of 150 birds in a flock died from acute FC.

Botzler (1991) reported that petechial hemorrhages in internal organs, liver necrosis, mucoid enteritis, and mucoid discharge from nares were related to FC infection.

Rimler and Glisson (1997) reported that chronic FC generally becomes suppurative. Infection with *P. multocida* often occurs in respiratory tract. Pneumonia is the common lesion in turkeys. Infection of conjunctiva and facial edema may be observed. Localized infection may also involve the hock joints,

foot pads, peritoneal cavity and oviducts. Also middle ear and cranial bones have been reported to result in torticollis.

Einum et al., (2003) reported an outbreak of acute FC in ring necked pheasants in Zeeland. At necropsy, hepatomegaly with multifocal cream-colored foci distributed throughout the parenchyma was observed in diseased birds. The spleen was enlarged up to three times its normal size and had a marbled appearance.

Kwon and Kang (2003) recorded that the gross lesions of FC in water fowl were multifocal necrotic foci in the liver with enlargement, petechial or ecchymotic hemorrhages on the heart, mucoid exudates in the duodenal mucosa and hemorrhagic enteritis.

Zhang et al., (2004) reported 2 outbreaks of FC in chickens in 1994 and 2002. Liver necrosis, fibrinous peritonitis, pericarditis, perihepatitis and pneumonia with abscess formation were observed macroscopically in the dead birds.

Shivachandra et al., (2005) described pathological changes in layer flocks due to FC outbreaks as general hyperemia, enlarged liver with focal necrosis and petechial haemorrhages on subepicardial and subserosal areas. Congestion of lungs and kidneys with distended tubules were also noticed.

Shilpa and Verma (2006) revealed that congestion of lungs, liver, spleen, kidney and heart and petechial/eccymotic hemorrhages on the surface of heart usually occur in *P. multocida* infection in chickens. The pericardium showed fibrinous pericarditis.The liver and spleen revealed mottling and congestion, spleenomegaly and occasionally necrotic foci in liver.

Herath et al., (2010)described the gross lesions induced by vaccinal strains A:1 of *P. multocida* in chickens at 14 day post-challenge as severe congestion,

hemorrhages, pericardial and peritoneum exudations, enlargement of spleen and liver, white necrotic foci over liver and pneumonia.

Kamaruzaman et al., (2015) recorded multifocal pin-point white spot lesions on the surface of the liver, petechial haemorrhage on the epicardial fatty tissue of the heart and also congested lungs in chickens infected with FC.

Mehmood et al., (2016) recorded the post-mortem lesions of FC in commercial layer flocks were cloudy air sacs, petechial hemorrhages on serosal membranes and necrotic foci on the liver. Moreover, large amount of viscous fluid in proventriculus and intestine with hemorrhages in sub-epicardium and sub-serosal layer of hollow organs and lungs were observed.

Samar (2017) found that the main gross lesions in chickens and ducks infected with FC were corn meal liver, fibrinous perihepatitis and fibrinous pericarditis. Swollen hock joint and thickened foot pad were grossly detected in ducks only.

2.5. Histopathological examination:

Glisson et al., (1989) stated that the microscopical lesions of *P. multocida* infection in Japanese quail revealed multifocal splenic and hepatic necrosis and interstitial pneumonia.

Gustafson et al., (1998) observed increased mortality with neurological and respiratory syndrome in layer flock infected with FC where the histopathological lesions were mainly associated with meningo-encephalitis and suppurative inflammation of the cranial air space.

Einum et al., (2003) found microscopically multifocal splenic and hepatic necrosis with intralesional rod-shaped bacteria due to FC infection in pheasants.

Kwon and Kang (2003) noticed multiple small hepatic necrosis, hemorrhages and necrosis in the myocardium and congestion and hemorrhages in

the mucosa and sub mucosa of the small intestine of dead Baikal teals where *P. multocida* was isolated.

Shilpa and Verma (2006) studied the histopathological changes of local isolates of *P. multocida* A:1 in chickens and found congestion, hemorrhages and mild degeneration of liver and necrotic changes involving groups of hepatic parenchymatous cells with prominent heterophilic infiltration. Congestion and hemorrhages in heart were seen, where the pericardium and myocardium were thickened with leukocytic infiltration and fibrinous exudates. Congestion of kidney with glomerular degeneration and hypercellularity, tubular degeneration, tubular necrosis and leukocytic infiltration were also noticed. Spleen revealed congestion hemorrhages, depletion of lymphoid elements and hyperplasia of reticuloendothelial cells with presence of bacterial colonies.

Hablovarid et al., (2009) described the histopathological lesions of vaccinal strain *P. multocida* A:1 in chickens. Congestion and hemorrhages of the internal organs were the most prominent features, with presence of viscid mucus in the lumen of the digestive tracts.

Samia (2009) found that the most prominent microscopic lesions of pasteurellosis in experimental chickens were observed in the liver, spleen and kidney. In the liver, degenerative changes of the hepatocytes, congestion of blood vessels with hemolytic RBCs in the lumen, focal heterophilc infiltration and coagulative necrosis were seen. The spleen showed lymphocytic depletion. Kidney showed focal interstitial nephritis with degenerative changes in the renal tubular epithelial cells.

Mona (2015) studied the histopathological changes of *P. multocida* in experimentally infected chickens and recorded inflammatory response of the mucosa of the respiratory tract with loss of cilia and epithelial necrosis. Lymphocytic necrosis and high depletion in white pulp of spleen were also observed. Dilatation and congestion of blood vessels of myocardium were

detected. Liver showed dilatation of portal vessels with few inflammatory cells infiltration.

Panna et al., (2015) found that the most prominent microscopic lesions of *P. multocida* in experimentally infected chickens were congestion with hemorrhages in heart, liver and spleen. Inflammatory cells in the pericardium of heart, and lymphocytic infiltration in central vein in liver were seen.

2.6. Isolation and identification of *P. multocida*:

2.6.1. Isolation:

Das (1958) described a selective medium containing aesculin, crystal violet and cobalt chloride for isolation of *P. multocida*.

Heddleston (1972) described the colonies of *P. multocida* on dextrose starch agar at 37ºC for 24 hr as strongly or weekly iridescent, sectored with variable iridescence or blue coloration. Iridescent colonies were circular, smooth, convex, translucent and glistening with tendency to coallese. Cells from these colonies are encapsulated and usually virulent. Blue colonies were similar to iridescent ones (smooth, slightly convex or flat, translucent and discrete). Cells from blue colonies were non capsulated and usually a virulent.

Waltman and Horne (1993) isolated *P. multocida*on5 % sheep blood agar. After overnight incubation at 37ºC, colonies characteristic of *P. multocida* were picked from the blood agar plates and inoculated into brain-heart infusion broth.

Moore et al., (1994) developed a selective media to isolate *P. multocida* from wild birds and environmental samples. The *P. multocida* selective enrichment broth and agar consisted of a blood agar medium at PH 10 containing gentamycin, potassium tellurite, and amphotericin B.

Christensen et al., (1998) incubated *P. multocida* aerobically at 37ºC for 24 hr on blood agar containing 5% citrated bovine blood. All field strains were stored at -80ºC since their original isolation.

Muhairwa et al., (2000) isolated *P. multocida* from web-footed birds and chickens using swabs which were streaked on a selective medium (tryptose blood agar base) to which 5% citrated bovine blood, 0.02% bacitracin and 1% neomycin were added). After overnight incubation at 37°C under aerobic conditions, colonies morphologically resembling those of *P. multocida* were subcultured.

Jonas et al.,(2001) characterized nine *P. multocida* isolates from avian cholera outbreak and plated each swab sample onto trypticase soy agar supplemented with 5% sheep blood agar and MacConkey's agar and inoculated into trypticase soy broth. The plates and broths were incubated at 37ºC for 18-24hr.

Pedersen et al., (2003) incubated *P. multocida* aerobically at 37ºC for 24 hr on blood agar, supplemented with 5% calf blood. Subculture was made on blood gar.

Nadodha (2004) isolated *P. multocida* on blood agar after incubation at 37ºC for 24 hr. Non haemolytic single colony was observed. Sub culturing on MacConkey's agar and incubation at 37ºC for 24 hr revealed no growth of *P. multocida*.

Masdooq et al., (2008) succeeded in isolation of *P. multocida* from lung samples of chickens aerobically incubated at 37ºC for 24-48 hr on blood agar containing 10 % sheep blood.

Sthitmatee et al., (2008) inoculated *P. multocida* in tryptose broth at 37ºC for 6 hr and then sub cultured it on dextrose starch agar (DSA) at 37ºC for 18 hr.

Balakrishnan and Parimal (2012) inoculated the heart blood and tissue samples of turkeys and chickens into brain heart infusions agar, blood agar, MacConkey agar and nutrient broth and incubated at 37^{o}Cwith 5 % Co_2 for 2 hr for the isolation of *P. multocida*.

Mohamed et al., (2012) inoculated *P. multocida* in brain heart infusion broth and incubated at 37^{o}C for 18-24 hr then subcultured it on sheep blood agar.

Rigobelo et al., (2013) were able to isolate *P. multocida* on tryptose blood agar base containing 5% citrated bovine blood, 0.02% bacitracin and 1% neomycin.

Bhimani et al., (2014) inoculated a loopful of tissue sample in brain heart infusion broth at 37^{o}C for 24 hr, then sub cultured on blood agar for isolation of *P. multocida* from emu.

2.6.2. Identification:

2.6.2.1. Cultural and morphological characteristics:

Glorioso et al., (1982) identified *P. multocida* as a Gram negative, non motile and non spore forming rod occurring singly, in pairs, and occasionally as chains or filaments.

Rimler and Roades (1987) found that *P. multocida* produced colonies of 1.5 mm in diameter, glistening and convex with entire edges on 5% horse blood agar while they were about 2 mm in diameter, iridescent and had pearl like appearance on DSA media.

Rebers et al., (1988) reported that *P. multocida* measures 0.2-0.4 × 0.6-2.5 µm, but tend to become pleomorphic after repeated subculture. A capsule can be demonstrated in recently isolated cultures, using indirect methods of staining. In tissues, blood, and recently isolated cultures, the organism stains bipolar. Pili have been reported and responsible for virulence.

Rhoades and Rimler (1990)a recorded that *P. multocida* was a Gram negative and non motile coccobacillus with small glistening colonies.

Roades and Rimler (1991) stated that the iridescence colonies of *P. multocida* are related to the presence of a capsule and the blue colonies were isolated from birds with the chronic FC.

Rimler (1994) recorded that *P. multocida* was bipolar when stained by Giemsa especially from primary isolation.

Patel (2004) studied the cultural and morphological characteristic of ten *P. multocida* isolates. All the test isolates were Gram negative, coccobacillary rods and produced non-haemolytic colonies on blood agar and no growth on MacConkey agar.

Shivachandra et al., (2005) showed that the *P. multocida* is a Gram negative, coccobacilli by Gram's stain and bipolar organisms when stained by Giemsa. Colonies of *P. multocida* on blood agar were small glistening, mucoid and dew drop like.

Tharwat (2008) described 22 isolates of *P. multocida* obtained from layers and 18 isolates from broilers as Gram negative, bipolar and coccobacillary rods. On blood agar, the organisms showed typical non-hemolytic, round, grayish and smooth or mucoid colonies. Some of colonies were iridescence, while the others were non iridescence. The organisms were non motile and produce no growth on MacConkey's agar.

Sarangi and Panda (2011) found that all *P. multocida* isolates were small, circular, glistening and dewdrop like colonies with no hemolysis on blood agar. There was no growth on MacConkey's agar. Bipolar organisms were showed in methylene blue and Gram's staining.

Balakrishnan and Parimal (2012) isolated *P. multocida* from chickens and turkeys on blood agar. The produced colonies were characteristics of dew drop,

mucoid and non haemolytic. No growth was observed in MacConkey agar. Morphological identification revealed Gram negative coccobacilli with bipolar staining.

Kumar et al., (2012) observed small mucoid and dew drop like colonies of *P. multocida* on blood agar medium after incubation at 37^{o}C for 18 hr and appeared as Gram negative coccobacilli when stained by Gram stain.

Levy et al., (2013) identified *P. multocida* isolated from chickens as Gram negative, non motile, non spore forming rod occurring singly or pairs and occasionally as chain or filaments.

Bhimani et al., (2014) showed that the *P. multocida* colonies on blood agar were non haemolytic, small, glistening, mucoid and dew drop like, and appeared as Gram-negative coccobacilli when stained with Gram's stain. No growth on MacConkey agar.

Mehmood et al., (2016) isolated *P. multocida* on blood agar after inoculation at 37^{o}C for 24 hr. The produced colonies were gray, viscous, mucoid, translucent and non hemolytic. Bipolar Gram negative coccobacilli were observed.

2.6.2.2. Biochemical reactions:

Murti (1971) studied 87 avian and 37 mammalian strains of *P. multocida* and showed that a large number of avian strains fermented both arabinose and sorbitol while few strains fermented only arabinose. Most of the mammalian strains fermented sorbitol but not arabinose. It was also found variability in fermentation of xylose and trehalose by both avian and mammalian strains.

Heddleston et al., (1972)b studied the biochemical reactions of 74 isolates of *P. multocida* associated with FC from free flying birds and commercial poultry. All isolates fermented (producing acid but not gas) glucose, galactose, fructose, mannitol, mannose, and sucrose, didn't ferment dextrin, inositol, inulin,

lactose, maltose, raffinose andrhamnose, some isolates fermented arabinose, dulcitole, sorbitole and xylose. All isolates produced indole and all except 2 produced detectable amount of hydrogen sulfide.

Heddleston (1976) reported that for 1268 cultures of *P. multocida* from various hosts, 29 physiologic tests were studied. 97-100% fermented galactose, glucose, mannitol, mannose, fructose, and sucrose, produced hydrogen sulfide and indole, and reduced nitrate, 6-91% fermented arabinose, glycerol, sorbitol, trehalose and xylose. Fermentation of dextrin, dulcitol, inositol, inulin, lactose, maltose, raffinose, rhamnose, and salicin, change of litmus milk, production of urease and hemolysin, liquefaction of gelatin were negative with 97 to 100% of the cultures. Of 200 cultures tested for catalase and oxidase, all were positive.

Aida (1980) studied the biochemical tests of 22 strains of *P. multocida* isolated from chickens, ducks and turkeys and found that most of the isolates (81-100%) fermented glucose, sucrose, fructose, mannitol and xylose, 4.5-18% fermented arabinose, maltose and dulcitol while all the isolates were negative for lactose fermentation, methyl red, change of litmus milk, production of hydrogen sulphide and hemolysin andliquifaction of gelatin. Nitrate reduction test, production of indol and ammonia, and urease activity were demonstrated by all isolates.

Calnek et al., (1997) stated that *P. multocida* organisms were positive for glucose and sucrose fermentation, indol, catalase and oxidase tests while were negative for gelatine liquefaction and urease test and usually negative for lactose and maltose fermentation tests.

Patel (2004) studied the biochemical characteristics of ten *P. multocida* isolates. All the isolates (100%) were positive for oxidase, catalase, indole production and nitrate reduction and fermentations of glucose, mannitol, sucrose and mannose; while were negative for citrate utilization and fermentation of maltose, arabinose, lactose, dulcitol, salicin and trehalose.

Zhang et al., (2004) identified 22 isolates of *P. multocida* from two FC outbreaks of layer chickens after biochemical identification. All the isolates were positive for oxidase, catalase andindole production, and fermentation of lactose, mannitol, sucrose, mannose, glucose, sorbitol and xylose; while were negative for urease and B-galactosidase and fermentation of arabinose, dulcitol, lactose, maltose and trehalose.

Shivachandra et al., (2005) studied the biochemical reaction of *P. multocida* isolated from FC outbreaks. All strains produced indole, positive for oxidase, catalase, and nitrate reduction test, while no reaction with gelatin liquefaction, Voges-Proskaur, citrate and methyl red. Glucose, galactose, fructose, mannitol and sucrose were fermented.

Pannaet al., (2015) reported that *P. multocida*type A strain isolated from FC cases in chickens was positive for catalase and oxidase, produced indol and fermented glucose, mannitol and sacrose.

Mehmood et al., (2016) studied the biochemical reaction of *P. multocida* isolated from layer flocks. The isolates were positive to oxidase, catalase, urease, production of indole and H_2S, nitrate reduction, gelatin liquefaction, citrate utilization, fermentation of glucose, arabinose, maltose, lactose, dulcitol, inositole and sucrose.

2.6.2.3. Mouse assay:

Mariana and Hirts (2000) tested 13 field isolates of *Pasteurella* from chickens and ducks in Indonesia. Five isolates were found to be pathogenic to mice after inoculation intra-peritoneal (I/P) with 1000 colony forming unit (CFU) of bacteria in 0.1 ml of trypticase broth (TB) and 2 were pathogenic for chickens when challenged with 10^6 -10^8 CFU/dose.

Sototdehnia et al., (2004) performed pathogenicity test of *P. multocida* serotype A1 in chickens and mice. Groups of chickens and mice were inoculated

intramuscular (I/M) and I/P to various concentrations of *P. multocida* broth culture, respectively. This strain was highly virulent for chickens; those exposed to only 7 CFU of the organism died in less than 24 hr. Groups of mice exposed to the virulent strain died during 48 hr post inoculation.

Shivachandra et al., (2005) tested the pathogenicity of 30 *P. multocida* isolates from two outbreaks in a single poultry farm. The pathogenicity test was carried out in three mice for each isolates by inoculating 0.2 ml of 18 hr old broth culture containing approximately 2.4 $\times 10^8$ CFU/ml by I/P rout and observed for 72 hr to study the mortality pattern. Each strain of the tested *P. multocida* was found to be pathogenic to mice, causing mortality within 24 hr, and the organisms were re-isolated from their heart blood, and the impression smear revealed typical bipolarity of the organism.

Tharwat (2008) performed pathogenicity test of *P. multocida* serotype 1 and 3 in chickens and mice. He found that the subcutaneous (S/C) inoculation in chickens induced high morbidity and mortality than the intratracheal (I/T) inoculation and the clinical signs were observed on infected birds 2 days after S/C inoculation, while delayed up to 3 days after I/T inoculation. All mice exposed to the tested isolates of *P. multocida*died within 24 to 48 hr post-inoculation.

Pors et al. (2011) studied the host response to infection by using of mouse model of *P. multocida* pneumonia. Twenty female mice were divided into four groups. Three groups were infected with one of three isolates of *P. multocida* isolated from clinical cases of chronic porcine pneumonia with necrotizing, suppurative and non-suppurative lesions, respectively. The fourth group served as uninfected controls. Mice were killed 24 hr post infection and samples were collected for bacteriology and histopathology for detection of *P. multocida*. It was found in the lung and spleen. Lung lesions were characterized by deposition of fibrin in alveoli and bronchioles, perivascular oedema, suppuration and necrosis. The cellular infiltration was mainly of neutrophils. Splenic neutrophilic

infiltration was also evident. Minor differences in the severity and nature of lesions were seen according to the isolate of *P. multocida* used for infection.

Balakrishnan and Parimal (2012) obtained 8 isolates of *P. multocida* which 4 were of chicken origin and 4 were from turkey origin. Pathogenicity test of *P. multocida* isolates were conducted I/P in Swiss Albino mice. They found that all isolates killed the mice in 24 - 48 hr. The dead ones were subjected to postmortem and reisolation of *P. multocida*.

Mohamed et al., (2012) inoculated 2 ml of 18 hr old broth culture of *P. multocida* containing 2.4×10^8 CFU/ml into each of 3 mice I/P and observed for 24-48 hr. Heart blood, liver and spleen impression smears from the dead mice revealed bipolar organism using Giemsa staining.

Naz et al. (2012) tested the pathogenicity of *P. multocida* in 6-weeks old Swiss albino mice. A total of six mice were used for each isolate. Mice were inoculated I/P with 0.1 ml of inoculum containing $0.3x10^8$ organisms/ml in sterile normal saline. Control mice were injected with 0.1 ml of sterile saline. All the field isolates killed mice within 24 to 36 hr post inoculation. Giemsa stained smears prepared from heart blood of dead mice revealed bipolar organisms.

2.6.2.4. Serotyping:

Carter and Bain (1960) found that *P. multocida* types A and D were the most widely distributed geographically. All of the organisms belonged to types A and D killed chickens when given in small doses.

Heddleston (1962) mentioned that the serogroup A of *P. multocida* was the major cause of FC, a highly contagious disease responsible for substantial economic losses to the poultry industry worldwide.

Baroutchieva and Feinhaken (1974) used gel precipitation test to serotype *P. multocida* strains isolated from cases of FC in turkeys, chickens, ducks and geese. The most common serotypes were *P. multocida* types 1 and 3.

Rhoades and Rimler (1987) reported that five antigenically distinct capsular groups of *P. multocida* A , B , D , E and F were found.*P. multocida* of serogroup A and D were the most common cause of FC in chickens and turkeys and type A was predominant. Out of the 246 studied strains , 166 were capsular group A , 4 were group B , 4 were group D, 14 were group F and 58 strains were non-encapsulated and not serogroupable.

Ireland et al., (1989) examined serologically 65 *P. multocida* strains isolated from chickens in Australia between1980-1987. The most common serotypes were of serotype 1 and 3 suggesting that these serotypes should be incorporated into a multivalent vaccine to obtain effective protection against FC.

Sander and Glisson (1989) studied the somatic serotyping of *P. multocida* isolates from six broiler flocks and found three of the cases were caused by serotype 1,3 and two by serotype 3,4 and one by serotype 3.

Rhodes and Rimler (1990)b serotyped 733 strains of *P. multocida* isolated from avian hosts during the period of 1976-88.The majority of isolates were serotype 3 (29%); 1 (18%); 3,4 (12%); and 3,4,12 (9%). The 733 strains had been isolated from 25 species of avian hosts; 400 (55%) were from turkeys. The most common serotypes of strains from turkeys were serotype 3 (38%); 3,4 (18%); 3,4,12 (11%); and 4 (4%).

Rhoades and Rimler (1991) identified 5 capsular serogroup D strains of *P.multocida* from avian hosts and found that avian capsular serogroup D strains were potential cause of FC.

Rimler and Glisson (1997) classified *P. multocida* into the serogroups; A, B, D, E, and F based on their capsular composition and 16 somatic serotypes based on lipopolysaccharide.

Boyce et al., (2000)stated that the antigenicity of the capsule of *P. multocida* could be used to identify five serogroups A, B, D, E and F. Disease predilection

was generally related to serogroup, where heamorrhagic septicemia strain belongs to serogroup B or E and FC strains to serogroup A.

Chawak et al., (2000) characterized11 indigenous isolates of *P. multocida* of avian origin in India. Serotyping of 11 isolates revealed that 3 isolates were A: 1,3 type and 2 each belonged to A: 1, A: 3, D: 1 and D: 3 serotypes .

Glisson et al., (2003) mentioned that the most important serotypes of *P. multocida* which cause FC were serotype 1,3 and 4 .

Kumar et al., (2004) identified the prevalence of *P. multocida* organisms in different animal and avian species in India. Out of 418 samples collected from different outbreaks suspected to be caused by *P. multocida*, a total of 206 bacterial cultures were identified as *P. multocida*. All the 206 cultures were isolated from different domestic animal species (cattle, buffalo, sheep, goat, pig and rabbit), avian species (chicken, duck, quail, turkey and goose) and wild animals such as leopard and deer. Serotyping of *P. multocida* cultures revealed the presence of various serotypes (A:1, A:3, A:1,3, A:4, B:2, D:1 and –:1) among the livestock population.

Arumugam et al. (2011) showed that 75% of *P. multocida* strains isolated from different domestic animal species and birds were belonged to serogroup A or B.

2.6.2.5. Molecular identification using polymerase chain reaction (PCR):

Townsend et al., (1998) designed oligonucleotide primers which have proved to be valuable in the development of PCR assay for rapid species and type specific detection of *P. multocida*. The primer pair was KMT1T7 and KMT1SP6 produced an amplification product unique to all analyzed *P. multocida* isolates.

Gunawardana et al., (2000) mentioned that repetitive extragenic palindromic sequence PCR (REP-PCR)and pulsed field gel electrophoresis (PFGE) using the enzyme ApaI were employed to characterize 95 isolates of *P. multocida* including 73 avian isolates from Australia and 22 from Vietnam. The majority of field isolates were capsular type A, with the predominant somatic serovars of 1, 3, 4 and 3,4.

Townsend et al., (2001) developed a multiplex PCR assay as a rapid alternative to the conventional capsular serotyping system of *P. multocida*. The serogroup-specific primers used in this assay were designed following identification, sequence determination and analysis of the capsular biosynthetic loci of each capsular serogroup. The multiplex PCR clarified the distinction between closely related serogroups A and F and constituted a rapid assay for the definitive classification of *P. multocida* capsular types.

Shivachandra et al., (2005) applied molecular methods for detection and identification of *P. multocida* strains involved in two separate FC outbreaks. A total of 12 and 1 strains of *P. multocida* obtained from 2 outbreaks were subjected to phenotypic and genotypic characterizations. Phenotypically all strains were similar, however, DNA-based technique by employing PCR assay were found to be highly specific and sensitive for rapid detection and differentiation of strains. All 30 strains gave amplicons of 460 bp and 1044 bp specific for *P. multocida* and capsular serogroup A in the multiplex capsular PCR typing system. The result also indicated that molecular methods of detection and typing were rapid in comparison with conventional method.

Kardoose and Kiss (2005) studied 2 cases of FC outbreak series occurring in goose and turkey flocks. Most strains isolated from epidemiologically related outbreaks showed genetic relatedness, as revealed by enterobacterial repetitive intergenic consensus(ERIC)-PCR and PFGE, suggested that the consecutive outbreaks were due to recurrence rather than reinfection.

Shivachandra et al., (2006) studied the prevalence of capsular and somatic serotypes among 123 *P. multocida* strains isolated from chickens (94), ducks (22), quails (4), turkeys (2) and gees (1) from different geographical regions of India. Ninety two of the isolates belonged to serotype A:1, the most prevalent serotype, A:3 , A:1,3, D:3 and F:3 had two isolates each. Only one isolate was positive for serotype A:4 and D:1 and twenty isolates were untyped. A multiplex capsular PCR assay generated amplicons of size 460, 1044 and 854 bp in 106 isolates identified as capsular serotype A, and 15 in serotype D and 2 in serotypes F, B and E were not detected. The present finding suggested that a multiplex capsular PCR may be suitable for the rapid identification of *P. multocida* serotyping during epidemiological studies of FC.

Shivachandra et al., (2008) employed REP-PCR, ERIC-PCR, and single primer PCR assays to characterize 66 strains of *P. multocida* serogroup A:1 isolated from avian species belonging to different regions of India. The PCR based amplification of repetitive regions of *P. multocida* is a rapid technique with good discrimination and can be employed directly for routine typing of field isolates from FC outbreaks.

Bhimani et al., (2014) characterized by multiplex PCR 22 isolates of *Pasteurellas pp* isolated from 42 dead bird suspected to have died from FC. All isolates were found to be of capsular type A.

Panna et al., (2015) applied a molecular method for detection of *P. multocida* type A from naturally infected chickens. *P. multocida* was isolated from 11.42 % (n = 4/35) samples. The isolated *Pasteurella spp*. was subjected to DNA based technique by employing PCR assay which was found to be highly specific and sensitive for rapid detection of strains. All isolates of *P. multocida* exhibited the amplification of PCR - amplicons of 440 bp. All of the 4 isolates were confirmed to be type A indicated by amplification of 511-bp size.

Mehmood et al., (2016) applied a molecular method for detection and differentiation of *P. multocida* strains isolated from 5 commercial layer flocks of Karachi, Lahore, Vehari and Toba Tek Singh, Bengladesh. The DNA of each bacterial isolate was amplified using universal and capsular serotype specific primers of *P. multocida*. The isolates showed amplicons of 590 and 1048 bps were declared as *P. multocida* type A.

2.7. Vaccination against *P. multocida*:

Avakian et al., (1989) examined the efficacy of several FC vaccines in broiler chickens at 12 and 21 weeks of age using live and killed vaccines; respectively. Some groups vaccinated with only live or killed vaccines, while others received a live vaccine at 12 weeks followed by a killed at 21. They found that birds that received the live Clemson University (CU) vaccine twice or once followed by a bacterin survived challenge. Birds that received killed vaccine only were significantly less protected (86%) whereas all unvaccinated controls died within 72 hr after challenge. Antibody titers were usually higher in birds received bacterins than in those received live vaccine.

Prantner et al., (1990) studied the pathogenicity of FC caused by two vaccinal strains (M-9 and CU) and field isolate (86-1913) of *P. multocida* (serotype A:3,4) in 7-week-old turkeys inoculated by an oculo-nasal-oral technique. Turkeys inoculated with strain CU and isolate 86-1913 developed

severe progressive bacteremia that began at 4 hr post inoculation (PI) and peaked at 16-20 hr PI, as well as greater histologic lesion scores for necrosis, heterophil infiltrates, and intralesional bacteria than turkeys inoculated with strain M-9. The mortality of turkeys inoculated with isolate 86-1913 was significantly higher than for turkeys received the two vaccinal strains.

Esmaily et al., (2003) studied the immunogenicity of various cell wall fractions of *P. multocida* vaccine strain serotype A:1 including sonicated antigen,

heat stable (HS) , lipopolysaccharide- protein complex (LPS-P),outer membrane protein (OMP), capsular (CAP) and potassium thiocyanate (KSCN) - extracted protein and compared with inactivated whole cell vaccine in *vitro* by ELISA and in *vivo* by challenge in immunized chickens. The results suggested that both OMP and KSCN-extract protein were more effective immunogens and could be good candidates for development of subunit vaccine substitute for current whole cell formalized vaccine against FC infection in chickens .

Fatma (2004) reported that two doses of *P. multocida* prepared bacterin induced good immunity (80-90%) against challenge with the *P. multocida* of homologous immunogenic type but low protection (10-30%) against heterologous challenge. She proved that the locally prepared polyvalent bacterins should be used in cases of FC outbreaks, and the capsular antigen plays a little role in immunization when compared with the somatic antigen.

Rahman et al., (2004) determined the efficacy of experimentally prepared formalin killed FC vaccine in Fayoumi chickens via different routes of vaccination. *P. multocida* (PM-38) serotype 1 (X-73) was employed for vaccine preparation. Each of the experimental chickens was challenged with a virulent isolate of *P. multocida* 3.8×10^8 CFU / ml per birds (I/M). All of vaccinated chickens were protected against virulent *P. multocida* infection but all unvaccinated control birds died within 72 hr of challenge. I/M (both primary and booster) route of vaccination was found more effective than S/C route of inoculation. The higher passive haemagglutination (PHA) antibody titer was recorded with I/M (222.86 ± 25.60) than S/C (111.43 ± 12.80) route of vaccinated groups of birds. The result revealed the fact that I/M followed by S/C inoculation route could be done for immunization against FC in chickens.

Chung et al., (2005) tested the protection induced by a capsular *P. multocida* strain A:1 (PBA930) against *P. multocida* heterologous strains in mice and chickens. I/M administration of PBA930 to mice stimulated significant

protection against X-73 strain and the heterologous P-1059 (A:3) strain, but not against challenge with P-1662 (A:4) one. No protection was observed when PBA930 was inoculated by I/P or S/C routes in mice. Significantly, the capsular strain PBA930 was able to induce protection against challenge with wild type X-73 strain in chickens.

Jabbri and MoazeniJula (2005) prepared an inactivated trivalent FC vaccine consisted of serotype 1, 3 and 4 *P. multocida* strains. They proved that such vaccine gave 70-100 % protection against challenge with homologous strains, and showed an increase in antibody titer after twice vaccination of 8 weeks old chickens.

Sthitmatee et al., (2008) reported that the recombinant adhesive protein (rcp39) of *P. multocida* strain P-1059 (serovar A: 3) was highly protective for chickens against FC after challenge-exposure with parental strain P-1059 or heterologous strain X-73 (serovar A: 1) compared to various kinds of vaccines.

Hablolvarid et al., (2009) studied thetype and severity of gross and histopathologic lesions induced by vaccinal strain (serotype A1) of *P. mulocida*. Ten, 4-week-old chickens were inoculated I/M with 75 CFU of (0.5 ml of 10^{-7} dilution) bacterium. All birds died in less than 16 hr without prominent gross lesions in different organs. Microscopical examination revealed congestion and hemorrhage. Moreover, large amount of viscid mucus were observed in digestive tracts.

Bitew et al., (2009) prepared and tested three adjuvated [Montanide ISA 50, Al $(OH)_3$, and AlK $(SO_4)_2$] formalin killed FC vaccine with three different kinds of bacterial loads (10^6, 10^9 and 10^{12}) from 3 local isolates of *P. multocida* on layer chickens. There was significant difference in geometric mean antibody haemagglutination inhibition (HAI) titer between bacterial loads. The dose level of 10^{12} CFU elicited highest antibody response followed by 10^9 and 10^6which was the least. This experiment used three kinds of adjuvants and found that, the

adjuvants had no significant difference in stimulating the immune response. This study also indicated that AlK $(SO_4)_2$adjvanted vaccine at bacterial dose of 10^{12} gave the better HAI titer in comparison with other adjuvants and bacterial loads vaccines.

Parvin et al., (2011) studied the humoral immune response (serum antibody titer) after vaccination with FC vaccine in different breeds of commercial birds (Synthetic - 10, White Rock - 10, Aseel - 7 andAseel × Rhode Island Red - 10) using PHA. Primary and booster vaccinations were done in four groups with FC vaccine in a dose of 0.5 ml / birds using I/M route at 20 and 26 weeks of age, respectively. All of the vaccinated birds showed significantly higher humoral immune response after primary and booster vaccinations. However, no significant differences were observed in antibody titers between breeds on different occasions of vaccination. It was appeared that breed variation had no significant effect on immune response to FC vaccine.

Tatum et al., (2012) examined the hypothesis that vaccination with the same recombinant filamentous hemagglutinin (rFHAB2) peptides derived from *P. multocida* P-1059 (serotype A:3) which protected turkeys against P-1059 challenge could cross protect turkeys against challenge with *P. multocida* X73 (serotype A:1). The results showed that vaccination with rFHAB2 peptides significantly protected turkeys against lethal challenge from both *P. multocida* serotypes.

Levy et al., (2013) reported that the oil adjuvanted *P. multocida* vaccine produced better immune response in chickens when boostering with the similar dose and rout at 15 day after primary vaccination, also 0.5 ml dose produced higher immune response against challenge infection and found to be safe.

Ali and Sultana (2015) measured the humoral immune response in chickens against formalin- inactivated alum- precipitated FC vaccine. It was found that serum samples possessing higher PHA titers after 15 days of primary

vaccination manifested a declining tendency at 30 day post vaccination (DPV) and rapidly fell down at 60 DPV. Boostering of vaccination at this stage elucidated a rapid increase of PHA titers ranging from 128 to 256 at 30 DPV and declined gradually from 60 DPV and reached a titer of approximately 8 to 4 at 180 DPV. Challenge infection was conducted with five randomly selected vaccinates of all the groups of birds along with unvaccinated controls after 15 days of secondary vaccination. FC vaccination conferred 100 % protection while all the unvaccinated control birds succumbed to such infection.

Akhtar et al., (2016) prepared a formalin killed FC vaccine from the isolated *P. multocida* strains and subjected for the determination of antibody titer in chickens aging 15 weeks either by injecting 1 ml (group A) or o.5 ml (group B). It was found that antibody titers in the birds of group A and group B were 4.513 and 4.07; respectively after primary vaccination, and 4.843 and 4.37; respectively after booster vaccination and most of the vaccinated birds were found to be survived after challenged with virulent strain of *P. multocida*.

Varinrak et al., (2017) studied the ability of recombinant outer membrane protein H (rOmpH) FC vaccine to produce cross protection against heterologous *P. multocida* strains. Chickens were divided to three experimental groups depending on bacterial strain challenge exposure as well as three control groups. Eight-week-old layers were immunized intranasally three times at three-week intervals. The chickens were challenged 4 weeks after the final immunization by inoculation with homologous strain X-73 or heterologous strains P-1059 (A:3) or P-1662 (A:4). The results revealed 70% to 100% of the chickens were survived after exposure to avian *P. multocida* strains and the vaccine could induce cross-protection against heterologous strains of *P. multocida*.

2.8. Enzyme-linked immunosorbent (ELISA) assay:

Marshall et al., (1981) compared serological results of ELISA and microtiter agglutination (MA) test for measuring humoral antibody responses of

turkeys received oral vaccination against FC. They found that ELISA was more sensitive than MA test.

Solano et al., (1983) developed ELISA assay to determine the humoral immune response of chicken against *P. multocida* and compared the results with indirect haemagglutination (IHA) test. They concluded that the antibody titers measured by ELISA was at least twice as sensitive as IHA.

Briggs and Skeels (1984) determined the antibody level of the vaccinated chickens injected with the CU vaccine strain of *P. multocida* using the ELISA. Serum samples were collected weekly for 3 weeks, and found that the antibody titer was increased to a maximum at about 10 days then slowly decreased.

Dick and Johnson (1985) reported that an ELISA was developed to detect antibodies in broiler breeders vaccinated via wing web with CU FC vaccine. It was found that birds vaccinated 2 or 3 times proved to be sufficiently immune to withstand a virulent challenge, while non vaccinated birds or vaccinated only once were not sufficiently immunized to withstand a virulent challenge.

Hofacre et al., (1987) reported that ELISA titer after vaccination with FC was greater than or equal to 1000 resulted in at least a 92% protection after virulent challenge, while 23% survival in non vaccinated.

Avakian et al., (1989) reported that antibody titers by ELISA test produced more protection in birds that received FC bacterin than in those received live vaccine.

Sacco et al., (1994) used ELISA test to measure the primary and secondary antibody responses of 671 turkeys of two genetic lines to Newcastle disease virus and *P. multocida* vaccines. Poults were vaccinated at 6 and 12 weeks of age, and blood samples were collected 3 weeks after each vaccination. They found that the higher serum antibody titers to *P. multocida* were at 15 weeks old.

Esmaily et al., (2003) used ELISA test to detect the serological immune response in chickens vaccinated by various cell wall fractions of *P. multocida*.

Sthitmatee et al ., (2008) determined the antibody titers of the immunized chickens with recombinant adhesive protein of *P. multocida* by indirect ELISA and the results indicated that the chicken anti-rCp39 sera reacted to whole cell lysate of parental or heterologous strains.

2.9. Antibiogram:

Rajini et al., (1995) studied the*in-vitro* antibiogram for 30 *P. multocida* isolates and found high sensitivity to chloramphenicole (96.6%), followed by doxycycline hydrochloride, chlortetracycline, nitrofurantion, penicillin and trimethoprim-sulfamethoxazole.

Aye et al., (2001) performed antibiotic sensitivity test for *P. multocida* of turkey origin. The majority of the isolates were susceptible to amikacin, ampicillin, ceftiofur, cephalothin, enrofloxacin, florfenicol, gentamicin, neomycin, novobiocin, oxacillin with 2% NaCl, sarafloxacin, tilmicosin and trimethoprim with sulphadiazine and resistant to clindamicin, penicillin-G, tiamulin and tylosin.

Jonas et al., (2001) performed antimicrobial susceptibility test for *P. multocida* isolates and found that all the isolates were susceptible to ampicillin and trimethoprim. Seven (78%) were susceptible to doxycycline and gentamicin.

Patel (2004) examined *in-vitro* the antibiotic sensitivity of ten *P. multocida* isolates. All isolates (100%) were found sensitive to enrofloxacin, flumequine, chloramphenicol and tetracycline, while nine isolates (90%) were sensitive to norfloxacin and cephalexin, and two isolates (20%) were sensitive to penicillin-G. All the isolates were found resistant to sulphadiazine while four isolates (40%) were intermediately resistant to penicillin-G.

Shivachandra et al. (2004) studied the antibiotic sensitivity of *P. multocida* avian strains to select an effective antimicrobial agent for controlling the disease in India. The strains of *P. multocida* were subjected to antibiotic sensitivity tests using 20 different antibiotics. The studies indicated that the strains were most sensitive to chloramphenicol (73.98%), followed by enrofloxacin (71.54%), lincomycin (64.23%), norfloxacin (61.79%) and doxycycline-HCl (56.91%). The majority of the strains were found to exhibit intermediate sensitivity. Absolute resistance was observed against sulfadiazine.

Khaled (2006) reported that *P. multocida* isolated from ducks were highly sensitive to gentamycin, norfloxacin and oxytetracyclin.

Sarangi and Panda (2011) studied the *in-vitro*antibiotic sensitivity of *P. multocida* isolates and found that fluoroquinolone group of antibiotics was highly sensitive, whereas strong resistance was observed against penicillin G and sulfadiazine.

Balakrishnan and Parimal (2012) isolated 8 *P. multocida* strains of avian origin and all were subjected to antibiotic sensitivity. All the strains showed sensitivity to enrofloxacin, ciprofloxacin, oflaxacin, gentamicin, amikacin, ampicillin and penicillin. Both turkey and chicken isolates showed sensitivity to oxytetracycline and doxycycline whereas showed resistance to chloramphenicol, poymixin B, trimethoprim, erythromycin and triple sulpha. It was suggested that antibiotics should be used preferably after performing antibiotic sensitivity test.

Mohamed et al., (2012) tested 10 isolates of *P. multocida* for their susceptibility to seven antimicrobial agents (amoxicillin, florfenicol, ciprofloxacin, tetracycline, streptomycin, doxycycline and trimethoprim-sulphamethoxazole). They observed that all the tested isolates were susceptible to ciprofloxacin, florfenicole, streptomycin and sulphamethoxazole with trimethoprim and with varying degree of sensitivity to other agents.

Shimaa (2016) carried out antibiotic sensitivity test for 14 *P. multocida* isolates against 18 antimicrobial agents using disc diffusion technique. The result indicated that serotype A1 isolates were sensitive to thiampheicol, sulfamethoxazole + trimethoprime, oxytetracyclin, streptomycin and neomycin while serotype A4 isolates were sensitive to thiamphenicol, sulfamethoxazole + trimethoprime, ciprofloxacin, amoxicillin, gentamycin and doxycyclin and untypable isolate was sensitive to thiamphenicol, sulfamethoxazole + trimethoprime, ciprofloxacin, and neomycin. All isolates were resistant to ampicillin, colisten, enrofloxacin, erythromycin, lincomycin, penicillin G, spiramycin and tetracyclin.

Ayman (2017) reported that *P. multocida* isolates were most sensitive to norfloxacin (85.72%), ciprofloxacin (71.44%) gentamycin(66%), doxycycline (62%) while the highest level of resistance to sulfadiazine (100%) was detected.