Faculty of Vet. Medicine

Veterinary Medical Journal-Giza (ISSN 1110-1423)
Faculty of Veterinary Medicine,Cairo University
Accredited from national authority for Quality Assurance and Accreditation
Giza, 12211, Egypt

Cairo University

Bacteriological and pathological studies on *Mycoplasma gallisepticum* in birds

*Attia,S.A.;[1]Mahmoud,A. M.;[2]Seida, A. A.;[1]Abed Elrahman.M. S.[3]**

[1]Dept. of Microbiol., Facult. of Vet. Med., ,Cairo University, Giza ,Egypt.
[2]Dept. of Pathol., Facult. of Vet. Med., ,Cairo University, Giza ,Egypt.
[3]Graduate student., Facult. of Vet. Med., ,Cairo University, Giza ,Egypt.

Abstract

***Mycoplasma gallisepticum* (MG) is the most important pathogen of avian Mycoplasmosis, in the present study One hundred and fifty positive serum samples for *MG* plate agglutination test (SPA) were collected .The included samples were one day old chicken (36 sample), broilers (40 sample), layers (38 sample), balady chicken (14 sample)and turkeys (22 sample). The samples were cultivated in mycoplasma media containing sterile filtrated swine serum to detect *MG*. all suspected colonies were identified by conventional method, growth inhibition test and PCR.**

In this studies culture methods detect MG from 35 and 23.33 percent of the suspected samples and Polymerase chain reaction confirmed the *nucleic acid* of the *MG* using universal and MG specific primers. Cumulatively PCR confirmed MG positive in (16%) of samples from 150 field samples collected during the study.

Tissue samples from the positive *MG* serological cases were examined histo-pathologically for showing lesions in the tissues. The clinical signs and pathologic lesions were much more significant in the diseased bird by *MG*.

The pathological changes in upper respiratory tract and lung showing large masses of caseous exudate in the air sacs, predominantly in the abdominal ones and egg. Peritonitis was observed in combined infection with CB. Thoracic air-sacs. Bilateral thoracic as well as abdominal air saculitis with caseous exudate were observed in severely affected combined cases. Histopathological examination of hepatic tissue, heart, trachea and lungs of broiler chicken showed Severe pathological changes varied from mild degenerative changes to severe inflammation with different types of inflammatory cells infiltration and/or necrosis in the affected organs.

Key words: *Mycoplasma gallisepticum*, pathological lesions, Respiratory disease, chicken.
*Corresponding author: msas_msas@hotmail.com; msas_msas@yahoo.com; Tel. 01116047099, 01063863106.

Introduction

Mycoplasma gallisepticum is (MG) producing avian respiratory Mycoplasmosis, an endemic infectious contagious disease with a large range of clinical signs, from asymptomatic till severe **(OIE, 2007)**.The losses generated by disease are a consequence of mortality, low eggs and meat production, decrease of fecundity and hatchability percent, the high cost of treatment, expenses with prophylaxis and control. The disease is included among the OIE diseases for which the international marketing is compulsory controlled. The initiation of avian mycoplasmosis in poultry may be due to vertical or horizontal transmission. Horizontal transmission usually occurs by direct contact between infected and susceptible birds. However, Indirect transmission via humans or fomites may play an important role because of possible persistence of *MG* in the environment **(Christensen *et al.*, 1994)**. MG spread may also occur by contaminated airborne dust, droplets, or feathers **(Ley and Yoder, 1997)**. Vertical transmission of MG has been successfully produced following experimental infection of susceptible chickens **(Yoder and Hofstad, 1965; Glisson and Kleven, 1984; Yoder and Hopkins, 1985)**. Egg transmission of MG in chickens is well documented **(Fabricant and Levine, 1963; Olson *et al.*, 1964 and Sasipreeyajan *et al.*, 1987)**. *MG* has been isolated from air sacs of infected 21-day old embryos **(Russel and Cottew, 1972)**.Culturing from the air sacs and yolk membranes of 18- day old embryos is claimed

1972).Culturing from the air sacs and yolk membranes of 18- day old embryos is claimed

to be a sensitive method for quantitating egg-transmission of MG **(Glisson and Kleven, 1984).** The present study was under taken to evaluate the prevalence of improve the MG from different species one day old chicks, broiler, layer, balady and turkeys detection methods of MG among the examined birds to be based on the use of PCR and serological methods. as well as detection of pathogenesis for MG using histopathological examination.

Material and methods

Samples:

For isolation, PCR and serological identification:

A total of One hundred and fifty samples(one day old chickes, broilers, layers, balady chickes and turkeys)were collected from different farms in El- Kaliobia, Eldakahlia, El behera and Beni-seuif governoratesand examined To study the incidence of MG in the respiratory tract.Samples from nasal cavity, ifra-orbital sinus, trachea, air-sacs and lung were collected from the examined birds.All samples were placed in mycoplasma broth, **(Zain& Bradbury, 1996)**.Serial dilutions of specimens in mycoplasma broth were done because the presence of specific antibodies or antibiotics or inhibitory substances in tissues may inhibit mycoplasma growth unless they are diluted out.

A total of One hundred and fifty serum samples were collected from the investigated birds to detect MG antibodies using rapid serum agglutination test and hem-agglutiation inhibition test **(OIE 2008)**. Using MG hyper-immune serum (Specific *Mycoplasma* antiserum of MG obtained from Intervet International BV Boxneer, Holland).

Isolation and identification of MG:

The specimens were inoculated mycoplasma medium to isolate MG. the inoculated medium was incubated at 37 ^{o}C in a moist 10% CO_2 incubator for 3- 5 days Kleven, 2003) . The suspected colonies were examined under inverted microscope to detect the characteristic fried egg colonies.

All isolates were identified using digitonin sensitivity (Freundt, 1983), glucose arginine hydrolysis (Fnske & Kenny, 1976) tetrazolium (**Aluotto, *et al* ,** 1970) and growth inhibition test (Clyde, 1964).

DNA extraction (Fan et al., 1995b) :

DNA extraction

One ml of sample cultured in Frey's medium was centrifuged at 10,000g for 20 min twice and the pellet was washed with 70 per cent ethanol. The pellet was resuspended with 50 µl of Tris EDTA buffer and boiled for 35 minutes to release the DNA. The extracted DNA was stored at – 20 °C until use.

Polymerase Chain Reaction Primers:

The following forward and reverse primers were used for the amplification of target sequence of "16SrRNA gene" (530 bp) of *MG.* Forward primer: 5'AACACCAGAGGCG AAGGCGAGG-3' Reverse primer: 5'-ACGGATTTGCA ACTGTTTGTA TTG G –3'.

The following mixture of materials wassubjectedto PCR in a thermal cycler (Eppendorff) as per the procedure by **Kiss*etal* (1997).** The amplified product was separated on 1.5 percent agarosegel. MasterMix: 25µl (dNTPs, Taq polymerase and PCR buffer) ; Forward primer: 1 µl (40 picomols); Reverse primer: 1 µl (40 picomols); DNA template: 2µl; DNase free water to make up to 50 µl.

Amplification of 16S ribosomal RNA gene (Kempfet al. 1993):

- The Mixture of the reaction was consisted of 5 µl of 10 X reaction buffer (promega), 1.5 µl of 0.25 mMMgcl₂.,1 µl of 10 mMdNTP mix (Sigma), 0.5 µl DNA of sample was added (containing 50 ng) and 1µl primer (containing 30 pmol of each). Then 0.5 µl of DNA Taq polymerase (promega) was added and the mixture was completed by sterile D.W. to 50 µl. Positive and negative controls should be used in each run. PCR was performed on progene programmable thermal controller (England). Amplification reaction was performed by heating the sample for 1 minute at 90$^{0}C$for initial denaturation step. After this step forty cycles were performed as follows: denaturation for 15 seconds at 95°C, annealing for 20 seconds at 60°C, extension for 15 seconds at 75°C.
- An additional cycle (95°c for 15 seconds, 60°c for 45 seconds and 75°c for 5 minutes) was included as a final step.The analysis of PCR amplified products was done according to

Sambrook *et al.*(1989) by using 10µl of the PCR amplified product which was mixed with 2µl of loading buffer and this mixture was electrophorsed through 2 % agarose gel, then

DNA was stained by adding ethidium bromide and was visualized by UV fluorescence , then photographed.

Histopathological examination:

Tissue specimens from trachea ,lungs heart and liver of birds were fixed in 10% neutral buffered formalin for routine histo-pathological examinations. The fixed samples were washed in tap water overnight and exposed to ascend concentrations of ethanol (70, 80, 90 and 100%), cleared in xylene and embedded in paraffin. Tissue slides of 5 µm thick sections were prepared and stained with hematoxylin and eosin (H&E). The histo-pathological preparation was performed according to **Bancroft and Stevens,(1996).**

Results

Clinical signs: The collected birds showed sneezing, rales, coughing, and exudates from nostrils and eyes. The birds had swollen sinuses, sub-orbital swelling, high morbidity, low mortality, low weight gain and decreased in egg production and hatchability.

Gross lesions: The diseased birds had catarrhal exudates in nasal passage and trachea, accumulation of caseous material in lungs and cheesy material in air sacs. The lesions in joints were not observed in any of the dead bird. The chronic macroscopic lesions such as fibrino-purulent exudates in nasal and para-nasal passages and in trachea were noticed. Massive airsacculitis and cheesy materials stick to the respiratory surface of the air-sacs were not uncommon Fig (1).

Cultivation on Mycoplasma media:

On broth media: A slight color change (acidity) appeared in the broth medium on the 4th day and first centered mycoplasma colonies were recognized on plates on the 5th day of incubation.

On agar media: Mycoplasma colonies were recognized on plates on the5th day of incubation.

The microscopic examination revealed that, colonies of Mycoplasma appeared with fried egg appearance with depressed center as shown in Fig (2). All media were showed same positive results. The suspected Mycoplasma colonies were identified as ***MG*** positive by ***MG*** cPCR; all Mycoplasma isolates were ***MG*** positive as showing in Fig (3). **Histo-pathological findings:**

The examined tissue showed the following histo-pathological changes, In liver, the lesions were varied from mild tissue reaction including congestion and degenerative changes of the hepatocytes to severe fibrinousperi-hepatitis and necrosis. The central veins and hepatic sinusoids appeared congested (Fig.4 A) together with perivascular oedema around some of the blood vessels (Fig. 4B).The hepatocytes showed marked vacuolar degeneration seems to be fatty change (Fig. 4C). Fibrino-purulent-peri-hepatitis was a common finding where the hepatic capsule was thickened and sub-capsular areas showed numerous mono nuclear inflammatory cells aggregation including lymphocytes and macrophages. Scanty heterophils infiltration was also noticed (Fig. 4D).The lesions in the heart were severe, fibrinous pericarditis, hemorrhages, oedema and infiltration with macrophages and lymphocytes were noticed (Fig. 5A).In some of the examined cases, marked congestion of the blood vessels and hetrophiles infiltration was a common findings (Fig. 5B). The inflammatory cells sometimes infiltrate the myocardium indicating myocarditis (Fig. 5C).In other cases, hemorrhagic myocarditis was a common finding (Fig. 5D).In trachea and lungs, tracheitis was a common finding and characterized by congestion of the blood vesselsand mononuclear inflammatory cells infiltration, desquamation of the epithelial lining and deciliation (Fig 6A) were common. Catarrhal tracheitis were noticed and accompanied with hemorrhage in subepithelial layer together with congestion (Fig. 6B). Fibrinous tracheitis were also noticed in some of the examined cases where large number of mononuclear cells infiltration, fibrinous exudate and oedema were observed (Fig. 6C).

with mucous exudate in the lumen (Fig. 6E). The affected bronchus appeared with desquamated epithelial cells(Fig. 6F)

Table (1): Isolation of Mycoplasma strains from examined birds.

Examined Birds	No. of examined samples	Positive Fried egg colonies	serology				PCR	
			RSA		HI			
			NO.	%	NO.	%	NO.	%
One day old chick	36	8	22	61.11	14	38.8	6	16.66
broiler	40	13	31	77.5	17	42.5	8	20
layer	38	7	28	73.68	13	34.21	5	13.15
balady	14	2	7	50	6	42.85	1	7.14
turkey	22	5	11	50	7	31.81	4	18.18
total	150	35	99		57		24	

Fig. (1): Infected chickens with *MG* showed air-sacculitis. The walls of air sacs are thickened and covered by fibrin, with accumulation of Large caseous masses in the abdominal air sacs.

Fig. (2): characteristic fried egg colonies of MG after 72 h.

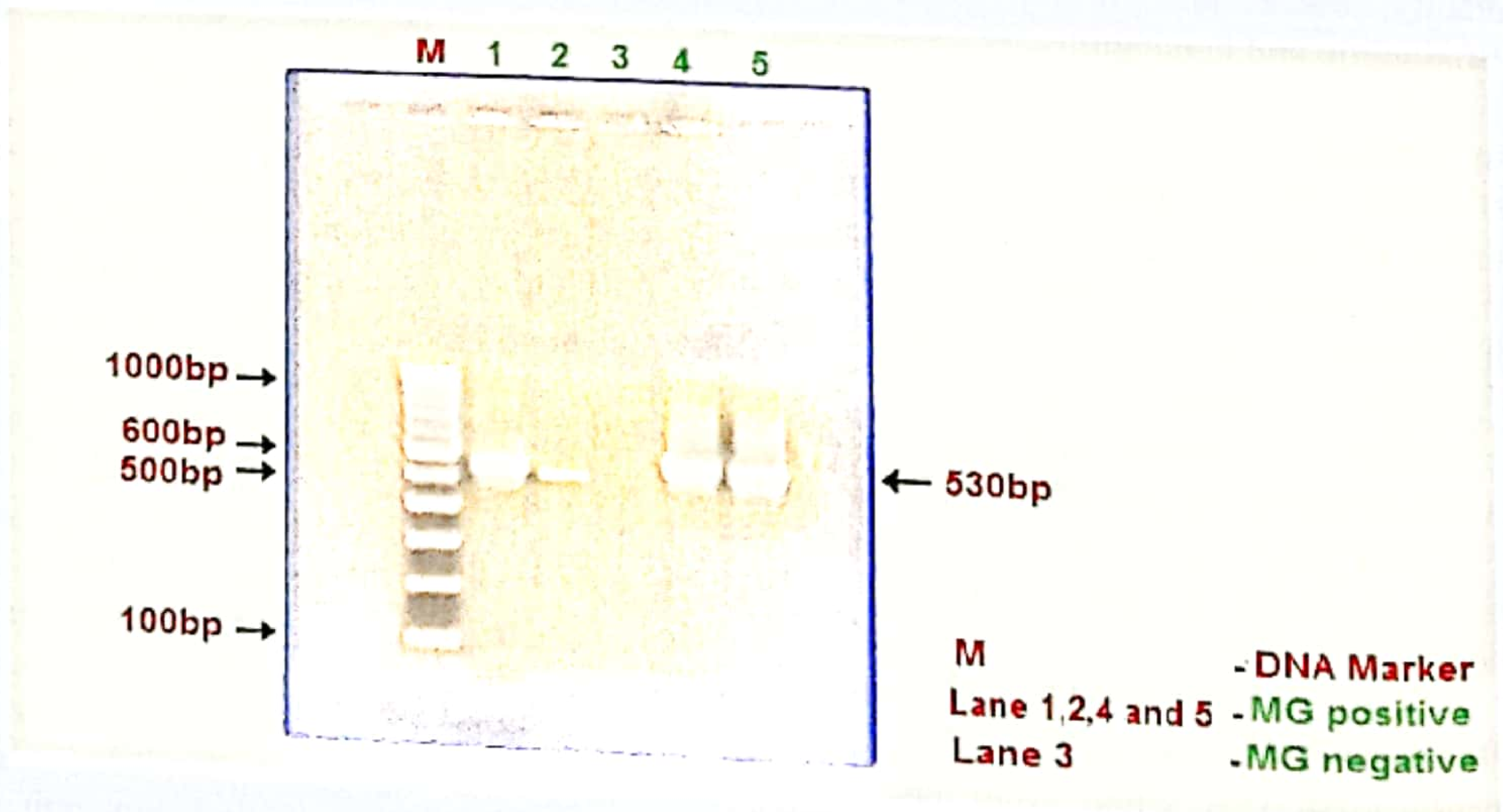

Fig. (3): Agarose gel electrophoresis showing amplification of 530 bp fragment specific for MG. *MG* – 530 bp PCR product on agarose electrophoresis.

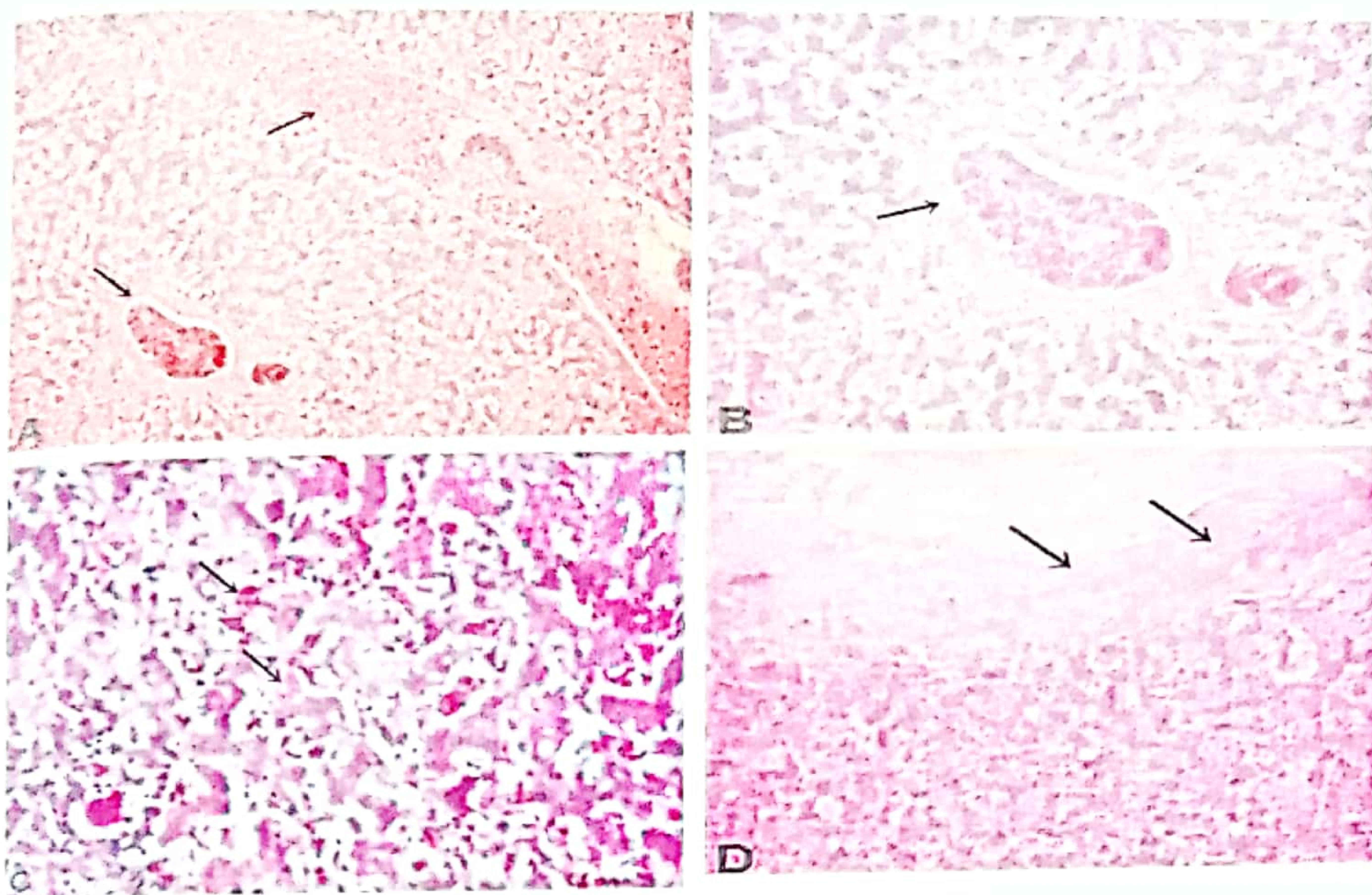

Fig.(4): Histo-pathological section of hepatic tissue of broiler chicken (H&E stain) showing:

a) Severe congestion of blood vessels (arrows). (x 100)
b) Higher magnification of the previous photo showing perivascular oedema (x200).
c) Vacuolar degeneration of hepatocytes and marked congestion of hepatic sinusoids (x400)
d) Perihepatitis with thickening hepatic capsule, fibrinous exudate and Inflammatory cells aggregation (x200).

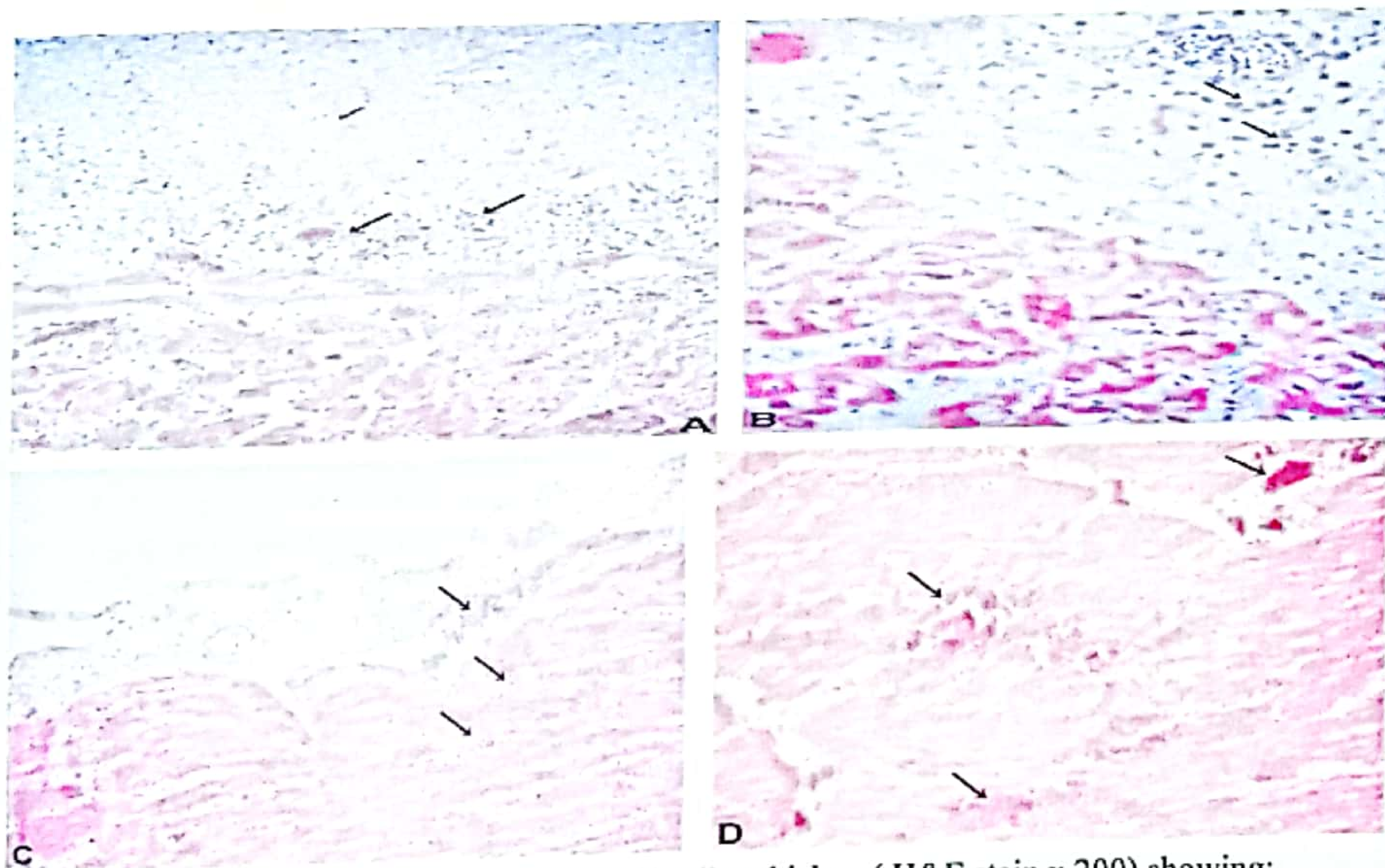

Fig.(5): Histo-pathological section of heart of broiler chicken (H&E stain x 200) showing:

a) Fibrinous pericarditis characterized by thickening of myocardium with fibrinous exudate (small arrows), hemorrhage and mononuclear inflammatory cells (Large arrows) together with oedema in the myocardium.
b) Mononuclear and heterophils (Arrows) infiltrating the inflamed pericardium with congested blood vessel.
c) Fibrinous pericarditis and myocarditis. large number of mononuclear cells infiltration in pericardium, epicardium and myocadium.
d) Hemorrhagic myocarditis showing aggregation of red blood cells and inflammatory cells infiltration .

CamScanner

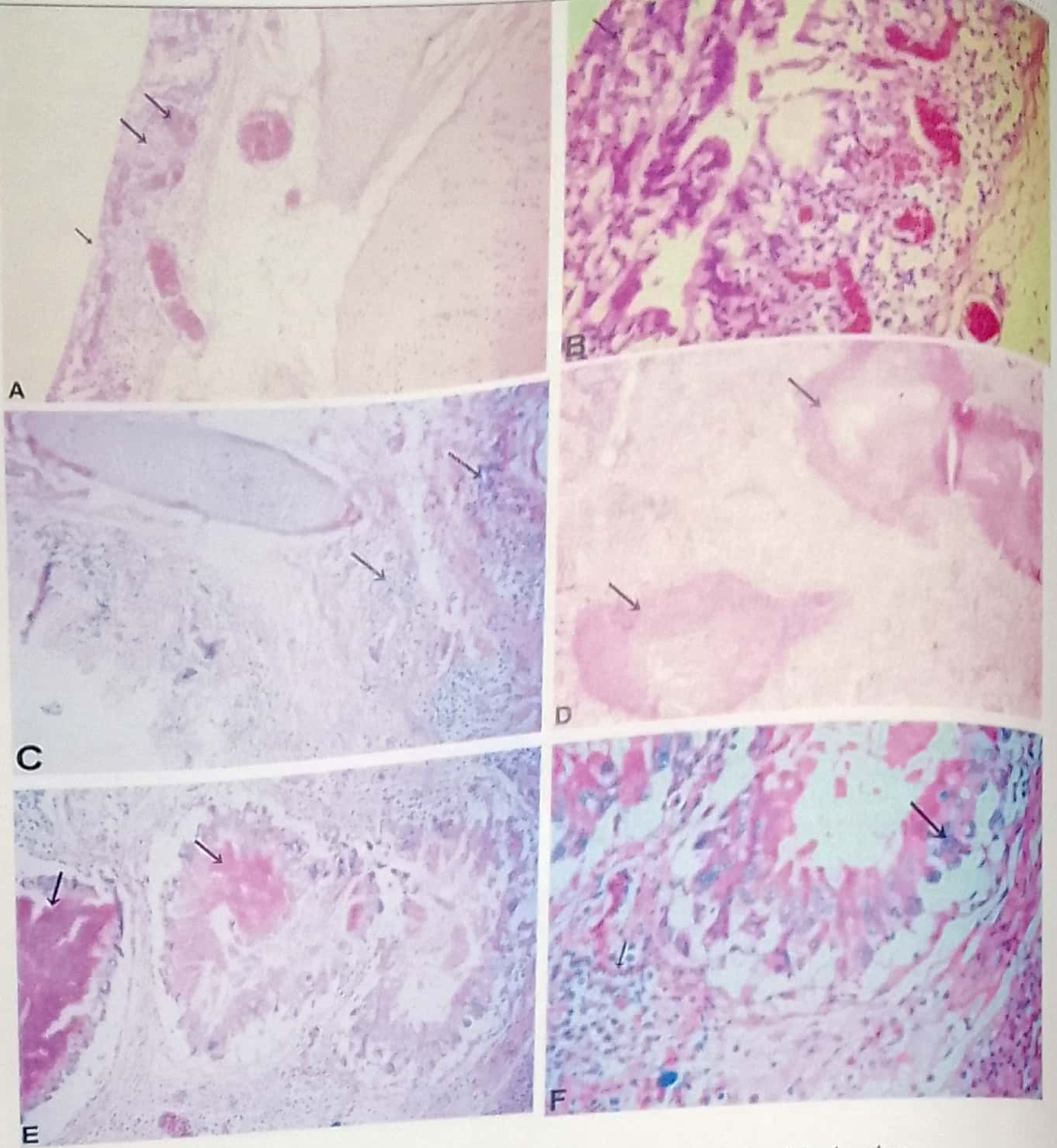

Fig.(6):Histo-pathological sections of trachea and lungs of broiler chicken(H&E stain) showing:

a) Tracheitis characterized by congestion of the blood vesselsand mononuclear inflammatory cells infiltration (large arrow), desquamation of the epithelial lining (Small arrow) (x 200).
b) Catarrhal tracheitis showing mucous exudate and inflammatory cells in the tracheal lumen (large arrow), hemorrhage in subepithelial layer with congestion and hemorrhage (Small arrow) (x400).
c) Fibrinoustracheitis showing large number of mononuclear cells infiltration (arrows), fibrinous exudate and oedema (x200) .
d) Severe fibrinous interstitial pneumonia (arrows), Severe fibrinonecrotic interstitial pneumonia (arrows), (x200).
e) Lung showing bronchopneumonia with mononuclear inflammatory cells infiltration in the interstitial tissue, desquamation of epithelial cells of the bronchioles and mucous exudate in the lumen (arrows).(x 400)
f) Lung showingbroncho-pneumonia characterized by mononuclear inflammatory cells infiltration in the interstitial tissue, desquamation of epithelial cells of the bronchus and mucous exudate in the lumen and oedema in the interstitial tissue (arrows). (x 400)

Discussion

Mycoplasma gallisepticum (MG) causes chronic respiratory disease (CRD) in poultry. The infected birds show sneezing, rales, coughing, and exudates from nostrils and eyes, swollen sinuses, suborbital swelling, high morbidity, low mortality, decrease weight gain (22-30 %), decrease feed conversion ratio (11-20%), egg production and hatchability ***(Shankar,2008)***.On necropsy of the diseased

birds, catarrhal exudates in nasal passage and trachea, accumulation of caseous material in lungs and cheesy material in air sacs were common lesions. Grossly, lesions in joints were not observed in any of the dead bird. In most of the cases, Mycoplasmosis is complicated with other microorganisms and environmental factors to enhance its severity **(Kleven, 2008).** The bacterium multiplies in lungs, trachea, and air sacs and rarely in sinuses, and causes chronic macroscopic lesions such as catarrhal exudates in nasal and para-nasal passages and in trachea. Massive air-sacculitis and cheesy materialsstickttotherespiratorysurfaceofthe airsac s.Incomplicatedcases, *E. coli* causes fibrino-purulent pericarditis, peri-hepatitis and turbid exudates in pericardial sacs **(Chanie*et al.*, 2009).** *MG* is small prokaryotes that lack a cell wall and are bound *Myaplasma* membrane **(Razin *et al.*, 1998).** Mycoplasma species grew well and showed turbidity with yellow coloration in Frey's broth and showed pure colonies like fried egg appearance on Frey's agar medium on day 10ofincubation at 37^0C and 10% CO2. Pathogenic avian mycoplasma species show similar cultural characteristics in mycoplasma broth and agar medium **(Frey's *etal.*,1968; Chanie*et al.*,2009).**

Mycoplasma species showed turbidity in the broth within three days post incubation at 37^0C in repeated broth culture. The bacterium ferments glucose of the medium and produces acid metabolites, which decreased pH of the medium and changed the color of phenol red to yellow. Mycoplasma species isolates were recovered from 39.3 per cent of the tracheal swabs, 15.9 per cent of tracheal tissues, 27.4% of lung tissues and 25 per cent of air sacs. *Mycoplasma* infection is prevailing in 2.1 per cent of the layers and 19.5 per cent of the broilers **(Heleili*etal.*,2011)** 38.1 per cent of layers, 31.3 per cent ofbroilers,14.3 per cent of breeders **(Saad-Ghariabeh and Al-Roussan, 2008)** and 33.3% of layers, 4.9 % of broilers and 30.5 % of broiler breeder **(Osman *et al.*,2009).**

PCR confirmed the nucleic acid of the *MG* from tracheal swabs (68.18%), tracheal tissues (42.47%), lung tissues (31.85%) and air-sacs (50%) using universal and MG specific primers. Cumulatively PCR confirmed MG from (49.74%) field samples collected during the study. PCR is a good technique for confirmation of MG from infected birds **(Nazarpak 2010).**

In this studies PCR confirmed the *nucleic acid* of the *MG* from 1-day old checks (6 +ve), broiler (8+ve), layer (5+ve), balady (6+ve), and turkey (4+ve) using universal and MG specific primers. Cumulatively PCR confirmed MG positive in (16%) Of samples from 150 field samples collected during the study.

PCR based *nucleic acid* detection is considered as an alternative method to that of conventional isolation technique **(Hess *et al.*, 2007).** MG is prevailing in 82.4 % layers, 64.8% of broiler breeders, and 17.1% of broilers are positive **(Osman *et al.*,2009)** and 25.8 % of the commercial farms are positive as confirmedt hrough PCR **(Faisal *et al.*,2011).**

Cultural methods of MG are laborious and time consuming, and could not isolate the organism and it is difficult in chronic cases of disease and medicated birds. New technique such as PCR is used for diagnosis of MG **(Ley *et al.*, 1993).** PCR proved more specific than culture method for identification of *MG* from field samples. Samples collected from medicated birds were PCR positive, whereas, the same samples were culture positive on specific medium **(Finklin and Kleven, 2006).** PCR and culture methods detect MG from 97 and 67 % of the suspected samples; respectively; in this study culture methods detect MG from 35 and 23.33 % of the suspected samples. whereas PCR show positive results on day 54 PI **(Kemf *et al.*, 1993 and 1994).** This difference of detection percentage could be due to fastidious nature of the organism and high sensitivity of PCR **(Chanie *et al.*, 2009).** Culturing of Mycoplasma is a gold standard technique but it could not isolate organism from chronic cases and medicated birds as MG concentration low in those conditions

(Hyman *et al.*, 1989) and antimycoplasmal substances, anti-serum and different types of inhibitors also decreased chances of isolation and time of isolation increases (Jordan,1979).Viable organism needed for success in isolation, alternatively, PCR detect the nucleic acid of MG even from medicated birds and frequent than culture (Kemf *et al.*, 1993). PCR amplifies DNA from dead o live pathogen (Josephson *et al.*, 1993). Higher percentage of positive samples are obtained by PCR(97%) tha that of culture method (67 %; Kemf *et al.*,1993).

Poor management, cold air currents during winter, vaccination, high-density poultry farming and rearing of multi age group chickens in the same premises, may ac tas potential causative factors for immunity break down against MG infection in chickens (Pradhan,2002).

Replication of vaccinal virus in chicken body post-vaccination, formaldehyde poisoning during spray, accumulation of ammonia gas due to poor ventilation and flow of dust particles in air due to very dry litter cause potential damage to tracheal epithelium of the chickens during inhalation which in turn support the multiplication of contaminating *Mycoplasma* infection (Carlile,1984).

Large Masses of caseus exudate in the air sacs predominantly in the abdominal ones (fig 1) were in accordance with the earlier findings of Gross (GROSS.1961), and Fabricant and Levine (Fabricant, and Levine.1963) who reported severe airsacculitis. These severe lesions might be due to the synergistic effect MG.

Also showed large masses of caseous exudate. Bilateral thoracic as well abdominal airsacculitis with caseous exudate were observed in severely affected combined cases.

Pathological studies, the gross lesions and microscopic tissue changes described above allowed to confirm the effect of *MG* on immunity and confirmed the bacteriological studies

It is concluded that *MG* Culturing method is laborious and time consuming and failed to detect *Mycoplasma* species from medicated birds and is less sensitive than that of PCR. PCR is rapid, sensitive and accurate method for diagnosis of MG from suspected cases.

Reference

Aluotto, B. B., R. G. Wittler, C. 0. Williams, and J. E. Faber.(1970): Tetrazolium. Standardized bacteriologic techniques for the characterization of *Mycoplasrna* species. Int. J. Syst. Bacteriol. 20:35-58

Animal and plant health inspection service (APHIS), USDA (2004): National Poultry Improvement Plan and Auxiliary Provisions. APHIS Publication 91-55-063.APHIS, USDA, Riverdale, Maryland, USA, 97–100.

Bancroft J D. and Stevens A.(1996): Theory and practice of hitopathological techniques.4th ed. London: Churchill Livingstone,

Chanie, M., T. Negash and S.B. Tilahun (2009): Occurrence of concurrent infectious diseases in broiler chickens is a threat to commercial poultry farms in Central Ethiopia. Trop.Anim.Hlth. Prod 41:1309-1317.

Carlile, F.S. (1984): Ammonia in poultry houses: a literature review. World Poult. Sci. J. 40:99-113.

Chanie, M., T. Negash and S.B. Tilahun (2009): Occurrence of concurrent infectious diseases in broiler chickens is a threat to commercial poultry farms in Central Ethiopia. Trop. Anim. Hlth. Prod. 41:1309-1317.

Charlton, B.R.; Bermudez, A.J.; Boulianne, M.; Eckroade, R.J.; Jeffrey, J.S.; Newman, L.J.; Sander, J.E. and Wakenell, P.S. (1996): In: Charlton BR, editor. Avian disease manual. Kennett Square, Pennsylvania, USA: American Association of Avian Pathologists. P.115-25.

Christensen, N.H.; Yavari, C.A.; Mcbai, A.J. and Bradbury, J.M. (1994): Investigations into the survival of *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and *Mycoplasma iowae* on materials found in the poultry house environment. Avian Pathol. 23:127–143.

CLYDE W.A., JR. (1983): Growth inhibition tests. *In*: Methods in Mycoplasmology, Vol. 1, Razin S. & Tully J.G., eds. Academic Press, New York, USA, and London, UK, 405–410.

Delaplane, J.P. and Stuart, H.O. (1943): The propagation of a virus in embrocated chicken eggs causing a chronic respiratory disease of chickens. Am. J. Vet. Res., 4:325-332.

Erno, H. and Stipkovits, L. (1973): Bovine Mycoplasmas. Cultural and biochemical studies. Acta. Vet. Scand. 14: 450-463.

F.(2000): lymphocytic infiltration in the chicken trachea in response to Mycoplasma gallisepticum infection. Microbiol. Reading.146:1223-1229.

Fabricant, J. and Levine P.P. (1963): Infection in young chickens for prevention of egg transmission of *Mycoplasma gallisepticum* in breeders.Proc. 17th World Vet. Cong., 1469-1474.

Faisal ,Z., A.Ideris, M. Hair-Bejo, A. Omar and T.G. Giap (2011): The prevalance of *Mycoplasma gallisepticum* infection in chicken from peninsular Malaysia. J. Vet. Anim. Adv.10(14):1867-1874.

Fan,H.H.; Kleven, S.H.; Jackwood, M.W.; Johanson, K.E.; Pettersson, B. and Levisohn,

Mycoplasmas by Polymerase chain reaction and restriction fragment length polymorphism analysis. Avian Dis., 39:398-407.

Finklin M and S.H. Kleven (2006): Evaluation of Diagnostic Methods for *Mycoplasma Gallisepticum* in chickens on 50g/ton tylosinin the Feed. Presented at the Georgia vet. med. asso. meeting, San Destin.

FREUNDT E.A. (1983): Culture media for classic mycoplasmas. *In* The Mycoplasmas, Vol. 1, Razin S. & Tully J.G., eds. Academic Press, New York, USA and London, UK, 127–135.

Frey, M.L., R.P. Hanson and D.P. Anderson (1968): Amedium for the isolation of avian mycoplasma. American J. Vet. Res. 29:2163-2171.

Gaunson J. E.; Philip, C. J .; Whithear L G.; Browning G Javed M T., Siddiqie M. (1991): coparative pathological studies Newcastle diseae, infectious bronchitis and infectious laryngotracheitis in chickens. Pakistan Vet.j.,11,82-85.

Glisson, J.R. and Kleven, S.H. (1984): *Mycoplasma galliseptic* vaccination: effects on egg transmission and egg production. Avian dis., 28: 406-415.

GROSS W B. (1961): Escherichia coli as a complicating factor of new castle disease vaccination. Avian Dis.,1961,5,132-134.

Heleili, N., B. Mamache and A. Chelihi (2011): Incidence of avian mycoplasmosis in the region of Batna, Eastern Algeria. Vet. World. 4 (3):101-105.

Hess, M., Neubauer C, Hackl R (2007): Inter laboratory comparison of ability to detect nucleic acid of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by polymerase chain reaction. Avian Pathol. 36 (2):127-133.

Hyman, H.C., S. Levisohn, D. Yogev and S.Razin(1989): DNA probes for *Mycoplasma gallisepticum* and *Mycopla smasynoviae* application in experimentally infected chickens. Vet. Microbiol. 20:323-337.

Jerstad, A.C. (1964): Isolation of *Mycoplasma gallisepticum* from fresh eggs and blood. Avian Diseases. 8:36-39.

Jordan, F.T.W. (1979). The mycoplasmas,1-48. J.G.Tully and R. T. Whitcomb (ed.),Avianmycoplasmas, 11. Academic Press, Inc.,NewYork.pp.432-496.

Josephson, K.L., C.P. Gerba and I.L. Pepper(1993): Polymerase chain reaction detection of non-viable bacterial pathogens. Appl. Environ. Microbiol. 59:3513-3515.

Kempf, I.; Blanchard, A.; Gesbert, F.; Guittet, M. and Bennejean, G. (1993): The polymerase chain reaction for *Mycoplasma gallisepticum* detection. Avian Pathol. 22(4): 739-750.

Kempf,I.; F. Gesbert; M. Guitte; G. Bennejean and L. Stipkovits (1994). Evaluation of two commercial enzyme-linked immunosorbent assay kits for the detection of *Mycoplasma gallisepticum* antibodies. Av. Pathol. 23(2):329-338.

Kiss I., Matiz K., Kaszaryitzky E., Chavez Y. and Johansson K F. (1997): Detection and identification of avian mycoplasmas by polymerase chain reaction and restriction fragment length polymorphism assay .Vet. Microbiol., 58,23-30.

Kleven, S.H. (2008): Control of avian mycoplasma infections in commercial poultry. AvianDis.52(3):367-374.

KLEVEN S H.(1998): Mycoplasmosis in **D.F. Swayne, J.RGlisson.M W. Jackwood, J.E. Pearson and W.M. Read** Eds. A laboratory manual for the isolation and identification of avian pathogens. 4thed.DennettSquare:Amererican Association of Avian Pathologists 74-80.

Lauerman, L.H.; Hoerr, F.J.; Sharpton, A.R.; Shah, S.M.andSanten, V.L. (1993): Development and application of a polymerase chain reaction assay for *Mycoplasma synoviae.* Avian Dis.. 37(3): 829-834.

Ley, D. H. ; Avakian, A. P. and Berkhoff, J. E. (1993): Clinical *Mycoplasma gallisepticum* infection in multiplier breeder and meat turkeys caused by F Strain: Identification by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, restriction endonuclease analysis, and the polymerase chain reaction. Avian Dis. 37:854-862.

Ley, D.H. and Yoder, H.W. (1997): ycoplasmosis/ *Mycoplasma gallisepticum* infection. InCalnek B.W. et al., (Eds.) Diseases of Poultry. 10th ed. Ames: IowaStateUniversity Press.194-207.

Nazarpak, H., Pourbakhsh, S.A., Charkhkar, S., Sheikhiand N. and Ashtari A. (2010): Isolation and detection o fMycoplasma synoviae from sero-positive rapid reaction broiler breeder flocks by polymerasechain reaction and culture methods. Vet. Res. Bull. 6,1:31-35.

OIE (2008): Avian mycoplasmosis (Mycoplasma gallisepticum, M. synoviae in Manual of diagnostic tests and vaccines for terrestrial animals. Chapter, 2, 3, 5.)

OIE (2007): World Organization for Animal Health [OIE] .Manual of diagnostic tests and vaccines [online]. Paris: OIE; 2004.Avian

Olson, N.O., Ken, K.M. and Campbell, A. (1964): Control of infectious synovitis. 13. The antigen study of three strains. Avian Diseases, 8: 209-214.

Osman, K.M., M.M. Aly, Z.M.S. Amin and B.S.Hasann(2009): *Mycoplasma gallisepticum*: an emerging challenge to the poultry industry in Egypt. Rev. Sci. Tech. Off. Int. Epiz. 28 (3):1015-1023.

Pradhan, M.A.M. (2002): Studies On Avian Mycoplasmosis: Prevalence, Isolation. Characterization and Antigenic properties. Ph.D. Thesis. Dept. of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Razin, S., D. Yogev, and Y. Naot (1998): Molecular biology and pathogenicity of mycoplasmas. Microbiol. Mol. Biol. Rev.62:1094-1156.

Russel, R.G. and Cottew, G.S. (1972): Isolation of *Mycoplasma gallisepticum* from embryonated chicken eggs. Aust. Vet. J. 48: 425.

Sambrook, J.;Fritsch, E.F. and Maniatis. T. (1989): Molecular cloning. A lab. Manual 2nd Ed., Cold spring Harbor laboratory, Press N Y.

Sasipreeyajan, J.; Halvorson, D.A. and Newman, J.A. (1987a): Effect of *Mycoplasma gallisepticum* Bacterin on egg-Transmission and Egg production. Avian dis. 31:776-781.

Shankar, B.P (2008): Common respiratory diseases of poultry. Vet. World.7:217-219.

Yoder, H.W. and Hofstad, M.S. (1964): Characterization of avian *Mycoplasma*. Avian Dis., 8:481-512.

Yoder, H. W. and Hofstad, M. S. (1965): Evaluation of tylosin in preventing egg transmission of *Mycoplasma gallisepticum* in chickens. Avian Dis. 9: 291-301.

Yoder, H. W. and Hopkins, S. R. (1985): Efficacy of experimental inactivated *Mycoplasma gallisepticum*oil-emulsion bacterin in egg layer chickens. Avian Dis. 29: 322-334.

Zain M.Z. and Bradbury J.M. (1996: Optimising the conditions for isolation of *Mycoplasma gallisepticum* collected on applicator swabs. *Vet. Microbiol.*,**49**, 45–57.

الملخص العربي

الميكوبلازما جاليسبتكم هو المرض الأكثر أهمية التي تصيب الطيو ر، وفي هذه الدراسة تم أخذ مائة وخمسين عينة مصل إيجابي لميكوبلازم اجاليسبتكم باستخدام اختبار التلزن (SPA). وكانت العينات التي قد تم تجميعها من كتاكيت عمر يوم (36 عينة)، الدجاج اللاحم (40 عينة)،الدجاج البياض (38 عينة)،الدجاج البلدى (14 عينة) ومن الرومي (22 عينة). و تم زرع العينات على مستنبتات بكتيرية والتي تحتوي علي سيرم الخنازير و اجراء الإختبارات البكتريولوجية للكشف عن الميكوبلازما جاليسبتكم و تم اجراء اختبارات بكتريولوجية و جزيئية مكثفة عليها تضمنت فحص المستعمرات و الفحص البيوكيميائي و كذلك التعريف الجزيئي باستخدام تفاعل البلمرة المتسلسل و كانت النتيجة هي عزل و تعريف ميكروب ميكوبلازما جاليسبتكم باستخدام جميع الطرق السابقة.

تم فحص عينات الأنسجة من الحالات الإيجابية المصلية والتغيرات المرضية في الأنسجة. وكانت العلامات السريرية والآفات المرضية أكثر أهمية بكثير في الطيور المريضة والمصابة بالميكوبلازما جاليسبتكم. وأظهرت النتائج وجود تغيرات مرضية في الجهاز التنفسي العلوي والرئة وكميات كبيرة من الافرازات في الاكياس الهوائية . وبالفحص الهستوباثولوجي تم فحص أنسجة الكبد والقلب والقصبة الهوائية والرئتين واظهرت التغيرات المرضية الشديدة نتيجة الإصابة بميكروب ميكوبلازما جاليسبتكم .