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RESEARCH ARTICLE

PREVALENCE OF MYCOPLASMA SYNOVIAE INFECTION IN BROILERS AND LAYERS WITH SPECIAL EMPHASIS TO IN VITRO ANTIMICROBIAL SENSITIVITY.

El shafey, Dina.Y.;Eissa, S.I.; Dardeer, M.A.; Abd El-Aziz, E,E; and Hassan .A.M
Mycoplasma Department,Animal Health Research Institute, Dokki, Giza, Egypt.

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Corresponding Author
El shafey.

Abstract

This work aimed to study the prevalence of Mycoplasma synoviae (MS) in some broiler and laying chicken farms. For this aim 103 samples (synovial fluid, bumbled foot exudates, lung , trachea, air sacs and tracheal swabs) were collected and examined for isolation of MS using specific medium. Three isolates were obtained; 2 from layers and 1 from broilers. Polymerase chain reaction technique (PCR) was adopted for detection of DNA of MS OIE primers. Minimal inhibitory concentration (MIC) was done to representative field isolate and a reference strain against some antimicrobials including (Tylvalosin(TVN), Enrofloxacin(Enro), Doxycycline(Dox), Lincomycin(linc), Tilmicosin(Til), Tiamulinefumirate(Tia), Amoxycline(Amox), TylosinTartarate(Tyl) and chloratetracycline(CTC). Tylovalosintartarate has the lowest MIC against field MS isolate.

Introduction:-

Four Mycoplasma species are recognized pathogens of Domestic poultry , causing entomic lose these species are Mycoplasma gallisepticum (MG) , Mycoplasma iowae (MI), Mycoplasma meliagides (MM) and Mycoplasma synoviae (MS)(Lierz et al.,2008). MG and MS are two common poultry pathogens that continue to significantly impact breeder , broiler and egg laying production . Mycoplasma infect approximately 90% of us egg laying birds and reduction in egg production are estimated as 140 million $ annually (Peebles et al., 2006). Mycoplasma synoviae is a major pathogen of poultry throughout the world, causing chronic respiratory disease and sinusitis in infected chickens and turkeys (Kleven, 1997), it may result in an infection ranging from subclinical to sever (Bencina et al., 2001; Anonymous, 2008). Clinical manifestations vary by strain, and some appear to have a greater tropism for synovial membranes or the respiratory tract than others (Kleven et al, 1975). The contaminated material with MS (dust, feathers and food) could infect chicks, sometimes after remarkably long silent periods (Corinne et al 2005. The success of control programmers depends on accurate and timely diagnosis of infected flocks. Therefore, a diagnostic assay with high sensitivity, high specificity, and a fast detection time is required for monitoring M. synoviae in poultry flocks, (Buntz et al 1986, Bencina et al 1988, Cookson et al 1994), rapid diagnosis is needed to prevent dissemination of infection and has traditionally been achieved by serological screening for antibodies or by culture of the organism. Traditional culturing is the least favored because the it is time consuming, slow growing , and fastidious nature of MS strains. Serology is much faster than culturing, but non-specific and cross-reactions between species, miss-interpretations due to recent vaccination, and cost are all disadvantages. MS strains can vary in antigenic make up and have the ability to alter the expression of major surface antigenic proteins affects the sensitivity and specificity of serologic monitoring systems (Adair et al 1990, Avakian et al 1990, Bradly et al 1988, Noormohammadi et al 1997). For these reasons, use of rapid and sensitive detection methods, like PCR method, can be advantageous (Fernandez et al 1993, Hyman et al 1989, Kempf et al 1993).

The emergence of M. synoviae infectious synovitis in both chickens and turkeys associated economic losses prompted the need for efficacious antibiotic treatments. In order to provide the optimum choice for antibiotic therapy, knowledge of the susceptibility profiles of available compounds was needed as underlined by

(Altwegg1995).So the objectives, of this work is to study the prevalence of MS in some broiler and layer chicken farms and to evaluate the most suitable antimicrobial agent contributing in control of the infection.

Material and methods:-

Sampling and cultures:-

A total of 103 samples were collected from broiler and layer farms for isolation of MS as shown in Table (1).Seventy three samples were collected from birds with respiratory manifestations, while 30 samples collected from birds with arthritis (in the form of bumbled foot ,swollen inter tarsal joint , foot containing gaseous exudate and gaseous sub cutaneous exudate with milled pectoral muscle atrophy (photo 2&3) .

Table 1: samples collected for isolation.

Samples	Broiler	Layer
Synovial fluid	20	0
Bumbled foot	8	2
Lung	20	5
Trachea	16	5
Air sac	7	0
Tracheal swabs	20	0
Total	91	12
	103	

Isolation and identification of MS:-

The collected samples are propagated in liquid and solid media specific for isolation of Mycoplasma as described by (Frey et al 1968).

Genus determination and biochemical characterization were carried out according to (Erno and Stipkovits 1973).

Primer selection for DNA amplification (OIE2008):-

Forward and reverse primer specific forMS approved by OIE was used ,which give characteristic fragment at 211 bp ,its sequence is as follows MS–F: 5'-GAG-AAG-CAA-AAT-AGT-GAT-ATC-A-3'MS–R: 5'-CAG-TCG-TCT-CCG-AAG-TTA-ACA-A-3'.

DNA Extraction:-DNA was extracted from samples according to (Pourbakhsh et al 2010.)

Amplification with specific primer (PCR):-Amplification was made according to (OIE PROTOCOL, 2008) which include The reaction mixture should be prepared in a separate clean area using a set of dedicated pipetters. For one50 µl PCR reaction the mixture is as follows:H2O Ultra-pure 35.75 µl,10 × PCR Buffer 5.00 µl, dNTP(l0 mM) 1.00 µl, F Primer (20 pmole/µl) 0.50 µl, R Primer (20 pmole/µl) 0.50 µl,Taq (5 U/µl) 0.25 µl,MgCl2 (50 mM) 2.00 µl, A 45 µl volume of the reaction mixture is dispensed into each PCR tube. The reaction mixture should be

Overlaid with a few drops of light weight mineral oil unless the thermocyler is equipped with a heated lid. The tubes are then taken to another clean area where the appropriate DNA sample (5 µl) is added to each tube. Positive and negative controls used in each run. The tubes are then placed in a thermal cycler for the following cycles: 40 cycles: 94°C for 30 seconds, 55°Cfor 30 seconds, 72°C for 60 seconds, 1 cycle (final extension): 72°C for 5 minutes and soak at 4°C. PCR products are detected by conventional 2% agarose gel electrophoresis, incorporating appropriate size markers, followed by examination under UV light. The PCR product for MS is 211 bp.Visualisation of the PCR products should be carried out in a separate laboratory area, well separated from all other steps in the PCR procedure.

Minimal inhibitory concentration (MIC) A:- The test was done using Sensititre commercial kit: Ref GBECO Trek diagnostic system, England ,andsome selected antimicrobials (Table 2) against Two strains of MS ;one reference stain (WVU1853 KLEVEN –Georgia-USA) and one filed isolates. According to (Hannan 2000).

Table 2: Drugs used for Minimum inhibitory concentration (MIC).

Drug	Producing company
Ciprofluxacine	INVESA-SPAIN
Doxycycline	GMP(certified Spain –EU)
Enrofloxacin	INVESA-SPAIN
Gentamycin	BMD
L incospectin	Pfizer -USA
Oxytetracycline	Pfizer -USA
Tylosine	ELANCO (USA)
Tilmicosine	ELANCO(Geneva)

Results and discussion:-

The poultry pathogens MG,MI, MM and MS have been isolated from a variety of different hosts (Bradbury 2006). M. gallisepticum and M. synoviae cause chronic respiratory disease (CRD), MScause infectious synovitis while air saculitis caused by both MG and MS with MM, however chronic and symptomatic infections are the most common and of a major concern ,due to the losses they cause (Keleven, 1997 and Morrow et al.,1990) . In our study 3 MS strains were isolated from 103 samples collected from birds showing lesions as in photo 2&3 that matched with what described by (Lockaby et al.,1998 and Moreira et al., 2014) who compared the pathogenicity of 6 stains of MS in broiler chicken 2 from layer hens and 1 from broiler chicken (photo 1A which illustrate the characteristic appearance of Mycoplasma, fried egg appearance and film & spot formation characteristic of MS photo 1B) and (Table 3). Isolation results are nearly agreed with those mentioned by (Eissa et al 2000)who succeeded to isolate 8 MS isolates out of 60 samples collected from inflamed turkey sinuses in a percent of 13.33%. Also (Hosseini Aliabad et al., 2012) recorded the isolation of 10 isolates out 32samples collected from poultry farms identified as MS. (Pourbakhsh et al., 2010) examined a total of 475 samples from broilers and obtained 160 positive samples for mycoplasma from which 85 isolates identified as MS. It is to be mentioned that Mycoplasma is a fastidious organism in isolation specially MS so molecular techniques are considered a fast and reliable method for identification of MS .Ehtisham-ul-Haque et al.,2010 cultured and isolated MS successfully 36 out of 85 (42.35%) samples. Whereas, PCR test has detected 84 (98.82%) positive cases.

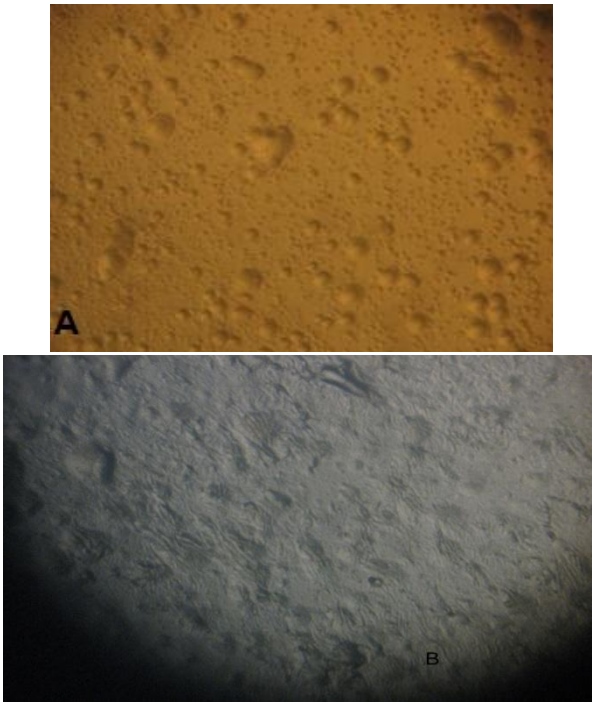

Photo1:(A);MS colonies (B) film and spot test of MS.

Photo 2: Leg; chicken swollen and inflamed knee and intertarsal joint anf foot containing gaseous exudate from where MS isolated.

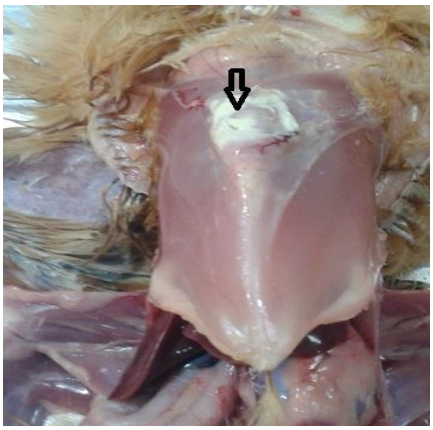

Photo 3: Keel; chicken gaseous sub cutaneous exudate (arrow) with milled pectoral muscle atrophy from where MS isolated.

Polymerase chain Reaction using primers described by OIE,2008 was performed to DNA extracted from all samples resulted in 25 positive sample out of 103(24.2%) (Photo 4) ,from which the percentage was 30% from bumbled foot exudate ,45% from synovial fluid of arthritic joint , and 65% of tracheal swabs Table (4). In present study, the possible reason for lower culture identification as compared to PCR assay might be due to the fastidious nature of MS colonies; bacterial overgrowth of non-pathogenic mycoplasma species (Salisch et al., 1999); low numbers of cells on the swabs (Zhao and Yamamoto, 1993), and samples collected from birds treated with antibiotics (Moalic et al.,1997). These findings have also been supported from the observation of (Bradbury 1998) that, the diagnosis using isolation is slow and laborious with reports of "atypical" strains of M. gallisepticum and M. synoviae which are extremely difficult to recover from birds.

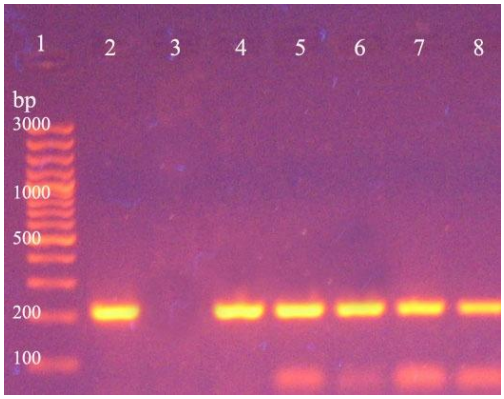

Photo 4: Electrophotogram of M. synoviae PCR amplicon by OIE Lane1: DNA marker100bp, 3 :negative control, Lane2: positive control Lanes 4 to 8 :Positive samples at 211 bp samples.

A PCR assay was developed and evaluated as a diagnostic test, because confirmation of M. synoviae infection by culture presents several practical challenges (Kleven et al 2008, Jordan et al 1979,Garcia et al 1996, Lauerman et al 1993, Lauerman,1998, Feberwee et al 2005). M. synoviae is fastidious organism with unique medium requirements; it requires 4 days up to 5 days for growth (Kleven et al 2008), even up to 28 days of incubation (Feberwee et al 2005), and initial cultures commonly contain other mycoplasmas, such as M. gallinarum and M.gallinaceum (Kleven 1998). In our investigation the higher MS incidence by PCR was illustrated from tracheal swab samples obtained from birds with respiratory manifestations which assure the important role played by MS in CRD similar finding explained by Bayatzadeh et al. (2011) who found in there study 76.8% of the samples taken proved positive for Mycoplasma genus and 55.9% of the samples taken proved positive for M. synoviae.

The broth culture method for MICs was undertaken rather than using solid media because the correlation between MICs and the inhabitant (Jordan and Horrocks ,1996).Two strains of MS (one reference and filed isolated strain) were subjected to MIC against 9 antibiotics (Table 2) .AivlsonSoluble contains tylvalosin(as tylvalosin tartrate) as a readily soluble, free flowing granule,Tylvalosin is a new macrolide antibiotic based on a highly active lactone ring. It has antibacterial activity against Gram-positive and some Gram-negative organisms and against mycoplasma by way of inhibition of protein synthesis in the bacteria cell (Table 5A). It is highly effective against a range of important diseases in pigs and poultry (ECO animal health). It was shown to have the best in vitro potency with 0.25 µg/ml ,while tilmicosin , Doxycycline , Timeline(TIA) and Tylosin is a macrolide antibiotic with a minimum inhibitory concentration (MIC) values ranging from 0.01 to 0.5 µg/ml for various susceptible bacterial and mycoplasmal pathogens (Jordan and Horrocks 1996, Hannan et al. 1997, Jordan et al. 1998, Salmon and Watts 2000).Tylosin tartrate was greater than Lincomycin ,Enrofloxacin and Chlortetracycline respectively (Table 5A&B) ,these results matches with what found by Pridmore2008,and Gautier-Bouchardon et al.,2002. However, Stanley et al. (2001) reported the occurrence of reduced affectivity of enrofloxacin for the treatment of an experimentally induced M. synoviae infection with a field isolate. The results of our study and the paper by Dufour-Gesbert et al. (2006) suggest an increase in the occurrence of quinolone- resistant M.synoviae field isolates. Landman et al.,2008 concluded based on the MIC breakpoints for M. synoviae , therapeutic failures are more likely to occur with quinolones than with oxytetracycline or tylosin. The MIC values found in this study further emphasize that M. synoviae infections should not be treated with quinolones. The application of quinolones may not only contribute to the selection of M. synoviae-resistant strains but may also affect other avian pathogens including zoonotic bacteria such as Campylobacte spp. Periodic assessment of the MICs of M. synoviae field isolates, and other bacteria in general, may contribute to a more rational and efficient use of antibiotics in the treatment of M. synoviae-affected flocks.

Our conclusion, PCR is a more rapid, effective, sensitive and inexpensive method than the standard culture technique, therefore, PCR can be an alternative method for traditional culture toward the detection of M. synoviae in broiler chicken farms ,also macrolides still have acceptable inhibitory effect on MS by MIC .

References:-

1. **Adair, B.M., Burns, K., McNulty, M.S., and Todd, D. (1990).** A study of ELISA systems incorporating pooled viral and mycoplasma antigen preparations for antibody screening of chicken sera. Avian Pathology 19: 263-278.
2. **Altwegg, M. (1995).** General problems associated with diagnostic applications of amplification methods. Journal of Microbiological Methods, 23, 21-30.
3. **Anonymous, (2008).** Avian Mycoplasmosis. In: Manual of diagnostic tests and vaccines for terrestrial animals (Mycoplasma gallisepticum, M. synoviae), Part.2,Section.2.3, Ch.2.3.5. OIE http://www.oie.int/eng/normes/mmanual/2008/ pdf/2. 03.05_%20avian_myco.pdf. Accessed on September 15, 2010.
4. **Avakian, A.P. and Kleven, S.H. (1990).** The humoral immune response of chickens to Mycoplasma gallisepticum and Mycoplasma synoviae studied by Immunoblotting. Veterinary Microbiology 24: 155-169.
5. **Bayatzadeh1, M.A., Pourbakhsh, S.A., Homayounimehr, A.R., Ashtari, A.,Abtin, A.R. (2011).** Application of culture and polymerase chain reaction (PCR) methods for isolation and identification of Mycoplasma synoviae on broiler chickenfarms.Archives of Razi Institute, Vol. 66, No. 2, December 87-94 .
6. **Bencina, D., Tadina, T. and Dorrer, D. (1988).** Natural infection of ducks with Mycoplasma synoviae and Mycoplasma gallisepticum and mycoplasma egg transmission. Avian Pathology 17: 441-449.

7. **Bencina D., Drobnic-Valic, M., Horvat, S., Narat, M., Kleven, S.H. and Dovc, P. (2001).** Molecular basis of the length variation in the N-terminal part of Mycoplasma synoviaehemagglutinin. FEMS MicrobiolLett, 203: 115-123.
8. **Bradbury,J.M (2006).**Mycoplasmas :a century and not out .Turkeys,54,9-11.
9. **Bradbury, J. (1998).** Identification of mycoplasmas by immunofluorescence.In R. Miles & R. Nicholas (Eds.), MycoplasmaProtocols , 1st ed. (pp. 119 _/126). Totowa: Humana Press.
10. **Bradley, L. D.,. Snyder, D. B. and Van Deusen, R. A. (1988).** Identification of species-specific and interspecies-specific polypeptides of Mycoplasma gallisepticum and Mycoplasma synoviae. Am. J. Vet. Res. 49:511–515.
11. **Buntz, B., Bradbury, J. M., Vuillaume, A. &Rousselot-Paillet, D.(1986).** Isolation of Mycoplasma gallisepticum from geese. Avian Pathol 15, 615–617.
12. **COOK5ON, K. C., and SHIVAPRASAD. H. L. (1994).** Mycoplasma gallisepticum infection in chukar partridges. Pheasants, and peafowl. Avian Diseases 38: 914-921.
13. **Corinne MAROIS, Jean-Paul PICAULT, Marylène KOBISCH and Isabelle KEMPF (2005).** Experimental evidence of indirect transmission of Mycoplasma synoviae Vet. Res. 36,759–769.
14. **ECO animal health data sheath ,** Website: www.ecoanimalhealth.com and www.aivlosin.com or www.valosin.com.
15. **Ehtisham-ul-Haque S, Rahman SU, Siddique M, Qureshi AS (2011):** Involvement of Mycoplasma synoviae in respiratory distress cases of broilers. Pakistan Veterinary Journal 31, 117–119.
16. **Eissa,S.I. ; Dardeer ,M.A and Abo-Norag,M.A (2000).** Application of sodium Dodecyl Sulfate-Polyacrylamide Gel electrophoresis (SDS-Page) For Identification of Mycoplasma infection in Turkeys with special Reference To Treatment. Vet.Med.J.,Giza. Vol.48, No.197-206.
17. **Erno,H and Stipkovits,L.(1973):** Bovine Mycoplasmas culture and biochemical studies. Acta.VeterinariaScandinavica, 14: 436- 449.
18. **Feberwee, A. Mekkes, D.R. de Wit, J.J. Hartman, E.G. and Pijpers A. (2005).** Comparison of culture, PCR, and different serologic tests for detection of Mycoplasma gallisepticum and Mycoplasma synoviae infections. Avian Diseases 49: 260-268.
19. **Fernandez, C., Mattson, J.G, Bolske, G., Levisohn, S., and Johansson, K.E. (1993).** Species-specific oligonucleotide probes complementary to 16S rRNA of Mycoplasma gallisepticum and Mycoplasma synoviae. Research Veterinary Science 55: 130-136.
20. **Frey, M. L, Hanson, R. P. & Anderson, D. R. (1968).** A medium for the isolation of avian mycoplasmas. Am J Vet Res 29,2163-2171.
21. **Garcia, M., Jackwood, M.W., Head, M., Levisohn, S., Kleven, S.H., (1996).** Use of species-specific oligonucleotide probes to detect Mycoplasma gallisepticum, M. synoviae and M. iowae PCR amplification products. Journal of Veterinary Diagnosis Investigation 8: 56-63.
22. **Gautier-Bouchardon, A. V., A. K. Reinhardt, M. Kobisch, and Kempf, I. (2002).** In vitro development of resistance to enrofloxacin,erythromycin, tylosin, tiamulin and oxytetracycline in Mycoplasma gallisepticum, Mycoplasma iowaeand Mycoplasma synoviae. Vet. Microbiol. 88:47–58.
23. **Ghaleh N. Golab Behbahan1, K. Asasi, A.R. Afsharifar and PourbakhshS.A. (2008).**Susceptibilities of Mycoplasma gallisepticum and Mycoplasma synoviea. International Journal of Poultry Science 7 (11): 1058-1064, 2008
24. **Hana C. Hyman,SharonLevisohn,DavidYogev, ShmuelRazin(1989).** DNA probes for Mycoplasma gallisepticum and Mycoplasmasynoviae: Application in experimentally infected chickens.VeterinaryMicrobiologyVolume 20, Issue 4, August, Pages 323–337
25. **HANNAN P C.(2000).** Guidelines and recommendations for antimicrobial minimum inhibitory concentration (MIC) testing against veterinary mycoplasma species. Vet. Res. 31 ,373–395.
26. **Hannan, P.C., Windsor, G.D., deJong, A., Schmeer, N., Stegemann, M. (1997).** Comparative susceptibilities of various animal-pathogenic mycoplasmas to fluoroquinolones. J AntimicrobChemother 41:2037-2040.
27. **Hosseini Aliabad, S.A., Pourbakhsh, S. A., Charkhkar S., BozorgmehriFard M. H. and Shikhi, N. (2012).** Molecular study and phylogenetic analysis of Mycoplasma synoviae isolated from poultry flocks from Mazandran Province of Iran. African Journal of Biotechnology Vol. 11(8), pp. 2124-2129, 26 January.
28. **Jordan FT, Forrestor, C.A, Ripley, P.H. and Burch, D.G. (1998).** In-vitro and in-vivo comparisons of valnemulin, tiamulin, tylosin, enrofloxacin, and lincomycin/ spectinomycin against Mycoplasma gallisepticum. Avian Dis 42:738–745.

29. **Jordan, F.T.W. and Horrocks B.K. (1996).**the minimum inhibitory concentration of Tilmicosin and Tylosin for Mycoplasma gallisepticum and Mycoplasma synoviae and a comparison of their efficacy in the control of Mycoplasma gallisepticum infection in broiler chicks. Avian Diseases 40:326-334.
30. **Jordan, F.T.W. (1979).** Avian mycoplasmas, in: J. G. Tully and R. F. Whitcomb (Eds.) The Mycoplasmas, Vol. II, Human and Animal Mycoplasmas, pp. 1-48 (New York,Academic Press).
31. **Kempf, I., Blanchard, A., Gesbert, F., Guittet, M. &Bennejean, G. (1993).** The polymerase chain reaction for Mycoplasma gallisepticum detection. Avian Pathology, 22, 739-750.
32. **Kempf, I., Gesbert, F., Guittet M. and Bennejean, G. (1994).**Mycoplasma gallisepticum infection in medicated chickens: comparison of diagnosis methods including polymerase chain reaction. Journal of Veterinary Medicine, 41, 597-602.
33. **Kleven S.H., Anderson D.P.(1971).** In vitro activity of various antibiotics against Mycoplasma synoviae, Avian Dis. 15 ; 551-557.
34. **Kleven, S.H. (2008).**Mycoplasma synoviae infection. In:Saif YM, Barnes HJ, Fadly AM, et al, eds. Diseases of Poultry. Ames, Iowa: Iowa State University Press,756-766.
35. **Kleven, S. H. (1998).** Mycoplasmas in the etiology of multifactorial respiratory disease. FtJult Science 77: 1146-1149.
36. **Kleven, S.H. (1997).**Mycoplasma synoviae infection in: Diseases of poultry,ed, Calnek BW,10th ed., pp.220-225.Iowa state university Press,Ames,IA.
37. **Kleven, S.H., Fletcher, OJ, and Davis, R.B. (1975)** Influnce of strain of Mycoplasma synoviae and rout of infection on development of synovitis or arisacculitis in broilers. Avian Dis.19:126-135.
38. **Lauerman, L.H., (1998).**Mycoplasma PCR assays. In:Lauerman, L.H. (Ed.), Nucleic Acid Amplification Assaysfor Diagnosis of Animal Diseases.M. gallisepticum and Mycoplasma synoviae infections. AvianDiseases 49: 260-268.
39. **Lauerman, L.H., Hoerr, F.J., Sharpton, A.R., Shah, S.M., Van Santen, V.L. (1993).** Development and application of a polymerase chain reaction assay for Mycoplasma synoviae. Avian Diseases 37: 829-834.
40. **Lierz M, Hagen N, Lueschow D, Hafez HM (2008).** Use of polymerase chain reactions to detect Mycoplasma gallisepticum, Mycoplasmaimitans, Mycoplasma iowae, Mycoplasma meleagridis and Mycoplasma synoviae in birds of prey. Avian Pathology, 375: 471- 476.
41. **Lockaby, S.B., Hoerr, F.J., Lauerman, H.L. and Kleven, S.H. (1998).** Pathogenicity of Mycoplasma synoviaein broiler chicken. Veterinary Pathology ,35 , 178 /190.
42. **MOALIC, P.Y., KEMPF, I., GESBERT, F. and LAIGRET, F. (1997).** Identification of two pathogenic avian mycoplasmas as strains of Mycoplasma pullorum. Int J SystBacteriol 47(1), 171-174.
43. **Mohammed, H. O., Carpenter, T. E. and Yamamoto. R. (1987).** Economic impact of Mycoplasma gallisepticumand Mycoplasma synoviae in commercial layer flocks. Avian Dis., 31:477-482.
44. **Moreira, F.A., Cardoso, L. and Coelho, A.C. (2014).** Decreased production in broiler breeders due to tendon rupture by Mycoplasma synoviae J HELLENIC VET MED SOC 2014, 65(2):109-114
45. **Morrow, C.J., Bell, I.G., Walker, S.B., Markham, P.F., Thorp, B.H. and Whithear, K.G. (1990).** Isolation of Mycoplasma synoviaefrom synovitis in chicken. Australian Veterinary Journal ,67, 121_/124.
46. **Noormohammadi A.H., Markham P.F.,Whithear K.G., Walker I.D., GurevichV.A.,Ley D.H., Browning G.F.(1997).**Mycoplasmasynoviae has two distinct phase-variable major membrane antigens, one of which is a putative hemagglutinin, Infect. Immun. 65; 2542–2547.
47. **OIE (2008).** Manual of Diagnostic tests and vaccine for Terrestrial Animals(Mammals,Birds and Bees),Avian Mycoplasmosis(Mycoplasma gallisepticum ,M.Synoviae).6thEdition,OIE.pages 482-496.http//www.oie.int/eng.
48. **Peebles ED, Basenko E.Y, Branton S. L, Whitmarsh S.K, and Gerard PD (2006).** Effects of an S6 Strain of Mycoplasma gallisepticum inoculation before beginning of lay on leukocytic characteristics of commercial layers. Avian Diseases, 48: pp 196-201.
49. **Pourbakhsh, S.A., Shokri1, G.R., Banani, M., Elhamnia, F.,Ashtari, A. (2010).** Detection of Mycoplasma synoviae infection in broiler breeder farms of Tehran province using PCR and culture methods. Archives of Razi Institute 65(2):75-81.
50. **Pridmore, A. (2008).** Antibacterial activity of tiamulin, valnemulin,tylosin and lincomycin against Brachyspiraand Mycoplasmaisolates: Determination of minimum inhibitory concentration (MIC). Report to Novartis.
51. **Robin, J. , Schultz, J., Hanning, I., Ricke, S.C. (2009).** Development of a real-time polymerase chain reaction assay for the simultaneous detection of Mycoplasma gallisepticum and Mycoplasma synoviae under industry conditions. Avian Dis. Mar;53(1):73-7.

52. **Salisch et al., 1999 Salisch, H., Hinz, K.H., Graack, H.D. and Ryll, M. (1998).** A comparison of a commercial PCR-based test to culture methods for detection of Mycoplasma gallisepticum and Mycoplasma synoviae in concurrently infected chickens. Avian Pathology, 27, 142_147.
53. **Salmon, S.A and Watts, J.L. (2000).** Minimum inhibitory concentration determinations for various antimicrobial agents against 1570 bacterial isolates from turkey poults. Avian Dis 44:85-98.
54. **Seifi ,S and Shirzad, M.(2012).** Incidence and risk factors of Mycoplasma synoviae infection in broiler breeder farms of Iran. Veterinary World J.;5(5):265.
55. **Stanley, H. Kleven and David P. A. (1971).** In vitro Activity of Various Antibiotics against Mycoplasma synoviae. Avian DiseasesVol. 15, No. 3 (Jul. - Sep.,), pp. 551-557
56. **ZHAO, S. and YAMAMOTO, R. (1993a).** Detection of Mycoplasmameleagridis by polymerase chain reaction. Veterinary MicrobiologVolume 36, Issues 1–2, July, Pages 91-97