ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 09, Issue, 10, pp.8854-8859, October, 2018

RESEARCH ARTICLE

PREVALENCE AND PATHOLOGY OF CHRONIC RESPIRATORY DISEASE IN BROILERS

***Poorna Chandhar, I., Madhu Swamy, Yamini Verma and Amita Dubey**

Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

ARTICLE INFO

Article History:
Received 25th July, 2018
Received in revised form
10th August, 2018
Accepted 24th September, 2018
Published online 30th October, 2018

Key words:
Chronic respiratory disease,
Histopathology,
Mycoplasma gallisepticum,
Polymerase chain reaction.

ABSTRACT

The study was conducted to determine the prevalence and pathology of Chronic Respiratory Disease (CRD) in broilers. Broiler carcasses (n=339) were examined for the gross lesions suggestive of chronic respiratory disease of which 36 birds (10.61%) had lesions strongly suggestive of CRD. Sixty pooled swab samples from nostrils, choanal cleft, trachea and air sacs were collected from broilers affected with respiratory infections and processed for Polymerase Chain Reaction (PCR). The overall prevalence of *Mycoplasma gallisepticum* (MG) in broilers with respiratory lesions was determined as 13.33% by PCR. Amongst these, 75% of birds found positive for MG were less than three weeks of age and 25% of samples positive for MG were from birds of seven to eight weeks of age. Microscopically, the histopathological findings noticed in the PCR positive CRD in trachea were hyperaemia, surface epithelial destruction, mucous gland hypertrophy, haemorrhage, mucosal thickening, infiltration of leukocytes and squamous cell metaplasia. Histopathological observations in lungs include congestion, haemorrhage, infiltration of cells, oedema, septal thickening, secondary bronchial mucosal hyperplasia, necrosis and denudation and respiratory atrial muscle thickening.

Citation: ***Poorna Chandhar, I., Madhu Swamy, Yamini Verma and Amita Dubey*, 2018.** "Prevalence and pathology of chronic respiratory disease in broilers, Ethiopia", *Asian Journal of Science and Technology*, 09, (10), 8854-8859.

Copyright © 2018, Poorna Chandhar et al. *This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

INTRODUCTION

Chronic Respiratory Disease (CRD) is a bacterial infection caused by one of the group of organisms known as pleuro pneumonia like organism (PPLO), but the particular organism directly associated with CRD is *Mycoplasma gallisepticum (MG), sometimes with secondary complications* (Ley, 2008). Chronic respiratory disease has been reported worldwide causing heavy economic losses in large commercial operations. The infection may be in-apparent or result in varying degrees of respiratory distress with slight to marked rales, difficulty in breathing and cough or sneezing. Morbidity is high and mortality low in uncomplicated cases (Bahatti *et al.,* 2013). Diagnosis of avian mycoplasmosis can be made on the basis of characteristic gross and histological lesions (Uddin *et al.,* 2010), on serological assays to detect antibody production and/or on isolation and identification of the organism (OIE, 2008). Detection of *Mycoplasma* by Polymerase Chain Reaction (PCR) has high specificity and sensitivity, which can amplify even very small amount of nucleotides that cannot be easily detected by other methods (Ramadass *et al.,* 2006 and Saritha *et al.,* 2010). The prevalence and pathology of Chronic Respiratory Disease (CRD) caused by *Mycoplasma gallisepticum* in broilers were studied.

Corresponding author: **Poorna Chandhar, I.,**
Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India.

MATERIALS AND METHODS

Samples were collected from twenty medium sized organised broiler flocks comprising between 5000-30000 birds and situated in a radius of approximately 50 miles.

Collection of sample

Sixty pooled swab samples from nostrils, choanal cleft, trachea and air sacs were collected at necropsy from broiler flocks which were suspected for avian mycoplasmosis and were subjected to PCR for the amplification of 16S rRNA of 185 bp as per, OIE Terrestrial manual, 2008, guidelines (OIE, 2008). All the birds found dead during the study period were examined for lesions indicative of mycoplasmosis. Tissue samples including the trachea and lungs were collected from thirty six broilers suspected for CRD based on the gross lesions.

Pathological studies

Gross pathology: Broiler carcasses (n = 339) were examined for the presence of gross respiratory lesions indicating CRD during post mortem. Overall occurrence of chronic respiratory disease was evaluated as proportion of bird necropsy. Chronic respiratory disease was suspected in birds with characteristic gross lesions as described by Ley (2008). The lesions

comprised sinusitis, catarrhal exudate in nasal passages, catarrhal exudate adherent to the tracheal wall with congestion and haemorrhages, bronchitis with accumulation of caseous material, congestion of lungs and foamy, cloudy airsacculitis with cheesy caseous exudates deposited over air sacs. Swabs from nostrils, trachea, choanal cleft and air sacs were collected from the birds suspected for CRD.

Histopathology: Sections of all trachea and lung tissues collected from broiler carcasses suspected for CRD based on gross lesions were processed for microscopic study (M. F. Gridley, 1960). Special staining such as Mayer's Mucicarmine staining and Masson's Trichrome staining were carried out in tissue samples for demonstration of mucin and connective tissue proliferation, respectively.

Molecular diagnosis of Mycoplasma gallisepticum: DNA was extracted from swab samples (three- five pooled) suspended in 1 ml of PCR grade PBS. The suspension was centrifuged for 30 minutes at 14,000 *g* at 4°C. The supernatant was then carefully removed with pipette and the pellet was suspended in 25 µl of PCR-grade water. The tube along with the contents was boiled for 10 minutes and then placed over ice for 10 minutes and then centrifuged at 14,000 *g* for 5 minutes. The supernatant thus obtained containing DNA, was used in PCR. The oligo primer (Table 1) specific to 16S rRNA of MG (OIE, 2008) was synthesized by Integrated DNA Technology (IDT) Inc. and utilized in the present study. Oligo primer supplied in freeze dried order form were reconstituted in Milli-Q water to the volume equivalent to the mass of primer (Mass/µg) and further diluted in Milli-Q water to give a final concentration of 10 pmol/µl. The PCR amplification of 16S rRNA gene was carried out in a final reaction volume of 25 µl. The PCR master mix for sixty samples was prepared and aliquot of 24 µl were added in each PCR tube. 1 µl genomic DNA was added in each tube, to make the final volume of 25 µl. Components of PCR reaction mixture are Ultra pure water (9.5 µl), PCR master mix (12.5 µl), F Primer (1 µl), R Primer (1 µl) and genomic DNA (1 µl). For every reaction, a positive control from genomic DNA of live vaccine Nobilis MG 6/85 (Merck Sharp and Dohme. Inc.) and a negative control were used to cross check any contamination of foreign DNA in reaction component.

The PCR tube was kept in a preprogrammed thermocycler (Veriti- 96 well thermocycler, ABI, USA) and thus standardized reaction programme including denaturation (94ºC for 30 seconds), annealing (57º C for 30 seconds), initial extension (72º C for 60 seconds) and final extension (72ºC for 5 minutes) was carried out. PCR amplification was confirmed by running 10 µl of PCR product mixed with 1 µl of 6X gel loading dye from each tube on 2% agarose gel at a constant voltage, 70-80 V for 60 minute in 0.5 X TAE buffer. Ethidium bromide was incorporated in 100ml of agarose gel at the rate of 5 µl of 1% solution. The amplified product was visualized as a single compact fluorescent band of expected size under U-V light and documented by gel documentation system (Syngene, Gene genius bio imaging).

Statistical analysis: Statistical analysis for association between gross CRD lesions and molecular detection of MG by PCR was determined by Fischer's exact (J. H. Mcdonald, 2014). IBM SPSS-20 (IBM Corp.) statistical software was used for statistical test. The *P* value less than 0.05 ($P<0.05$) was considered statistically significant for the test.

RESULTS

Gross lesions: Total 36 birds (10.61%) had lesions strongly suggestive for chronic respiratory disease. The gross lesions like sinusitis, exudate in nasal passages, caseous exudate adherent to the tracheal wall with congestion and haemorrhages (Fig. 1), airsacculitis with cheesy caseous deposits over air sacs (Fig. 2), congestion of lungs were characteristically observed in these birds (Table 2).

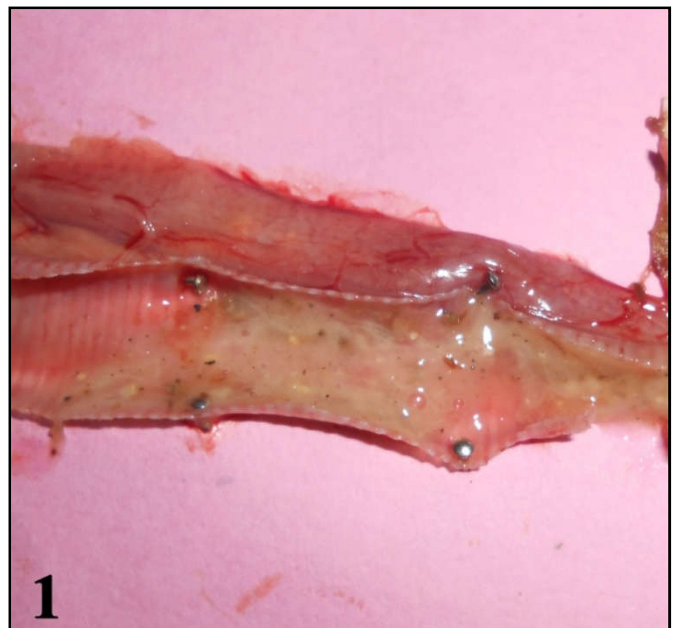

Fig. 1. Caseous exudate in tracheal lumen of three week old broiler

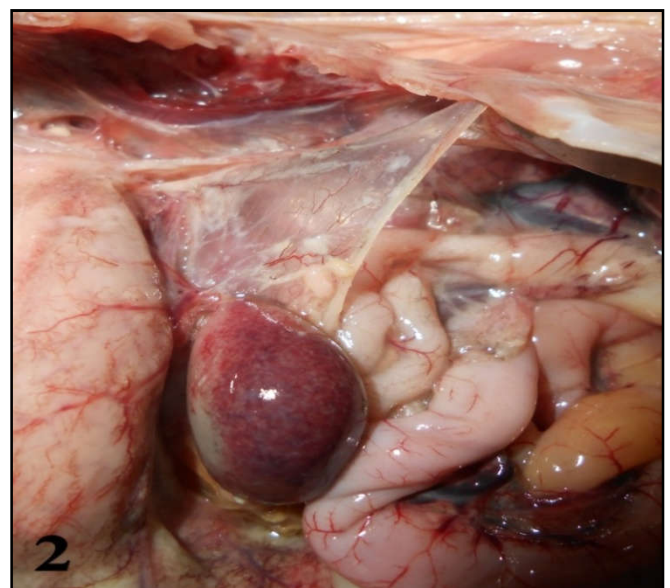

Fig. 2. MG positive broiler chick with severe airsacculitis and cheesy deposits over air sacs

Microscopic lesions: Sections of trachea and lung collected from the cases affected with CRD based on gross lesions were studied for microscopic changes. The percent distribution of microscopic lesions in trachea of affected broilers were hyperaemia (72.22%) (Fig. 3A), surface epithelial destruction (58.33%), infiltration of leukocytes in lamina propria (50.00%), mucous gland hypertrophy (44.44%) (Fig. 3B), haemorrhage (33.33%), mucosal thickening (27.77%) and squamous cell metaplasia (13.88%).

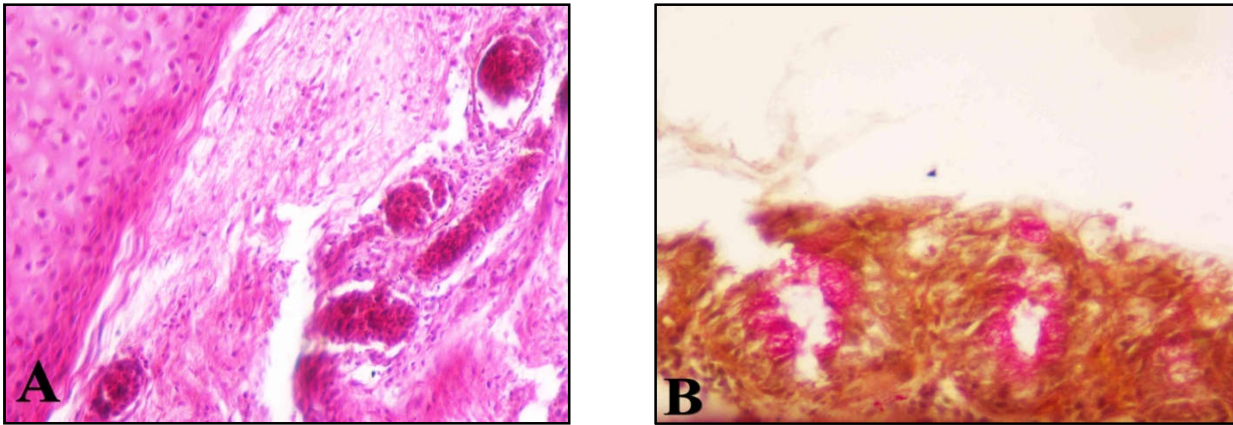

Fig. 3. Microscopic section of trachea with congestion and haemorrhages in lamina propria, H&E X 400 (A); Hypertrophied mucous gland with excess mucin deposition, Mayer's mucicarmine X 1000 (B)

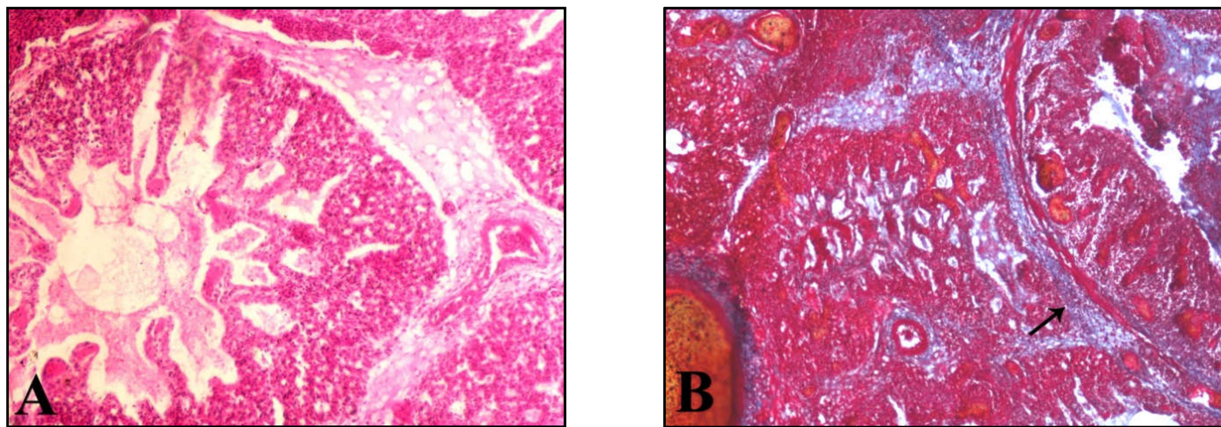

Fig. 4. Microscopic section of MG positive broiler lung with oedema and thickened interstitium, H&E X200 (A); Septal thickening (arrow). Masson's trichrome X100 (B)

L02, L04, L08: Positive sample
M : 100bp Ladder
L13 : Negative control
L14 : Positive control (Nobilis MG 6/85 vaccine)

Fig. 5. PCR amplification of 185 bp of 16S rRNA for identification of MG in broiler

Table 1. Detail of primer used in PCR

Primer	Sequence 5'-3'	Amplification region	Size
MG-14F	GAG-CTA-ATC-TGT-AAA-GTT-GGT-C	16S rRNA	185 bp
MG-13R	GCT-TCC-TTG-CGG-TTA-GCA-AC	16S rRNA	185 bp

Table 2. Summary of organwise distribution of gross lesions

S.No	lesions suggestive for CRD in different organs	No. of birds with lesions suggestive for CRD (n= 36)	Percent occurrence (%)
1	Sinusitis	28	77.77
2	Nasal exudates	36	100
3	Tracheal exudates	36	100
4	Congested lung	33	91.66
5	peri hepatitis	31	86.11
6	peri carditis	33	91.66

Table 3. Association between gross CRD lesions and molecular detection of MG by PCR

		Gross lesions		Total	*P* value
		Indicative for CRD	Not indicative for CRD		
MG detection by PCR	Positive	8	0	8	
	Negative	28	24	52	*P*= 0.017*
Total		36	24	60	

*Significant at P<0.05; MG- Mycoplasma gallisepticum

The lesions in trachea were further classified as acute catarrhal tracheitis (41.66%), acute haemorrhagic tracheitis (33.33%), chronic catarrhal tracheitis (13.88%) and fibrinous exudative tracheitis (08.33%), as per the microscopic observation. The overall findings noticed in lungs of broilers affected with CRD were congestion (91.66%), haemorrhage (83.33%), infiltration of cells (66.66%), oedema (33.33%) (Fig. 4A), septal thickening (33.33%), secondary bronchi mucosal hyperplasia (27.77%), necrosis and denudation (22.22%) and respiratory atrial muscle thickening (13.88%). Microscopically, depending upon the extent of involvement, the lesion was categorized as mild, moderate and severe congestion. The occurrence of mild, moderate and severe congestion was 12.12%, 42.42% and 45.45%, respectively. The occurrence of pneumonia was broadly classified as bronchopneumonia and bronchointerstitial pneumonia which accounts for 69.50% and 30.50%, respectively. Bronchopneumonia was further classified as haemorrhagic, catarrhal, necrotic and sero-fibrinous with 22.22%, 19.44%, 16.66% and 11.11% distribution, respectively. Bronchointerstitial pneumonia was observed in 30.50% cases. Special staining of microscopic section of lung with Masson's trichrome staining revealed thickened interbronchial septa (Fig.4B).

Prevalence of Mycoplasma gallisepticum by PCR: Of sixty samples, 8 samples were found positive for MG. In positive samples the PCR amplified product of 185bp of 16S rRNA of *Mycoplasma gallisepticum* was clearly visible (Fig. 5). The overall prevalence of MG was determined as 13.33% in broilers by PCR. Amongst these, 75% of birds found positive for MG were less than three weeks of age and 25% of samples positive for MG were from birds of seven to eight weeks of age. The average body weight of chicks at 2 weeks of age and infected with MG was determined to be 201.66 g, whereas average body weight of chicks at 3 weeks of age and infected with MG was 341.66 g. The body weight of chicks at 2 and 3 weeks was much less than the expected average ideal body weight. Association between gross CRD lesions and molecular detection of MG by PCR were determined by Fisher's exact test. The results are shown in Table 3. The Significant value, *P*= 0.017, indicates the positive association between gross CRD lesions and molecular detection of MG.

DISCUSSION

The overall prevalence of chronic respiratory disease based on gross lesions was found to be 10.61%. Earlier workers determined the prevalence of CRD based on gross lesions as 11.50%, 9.87%, 12.84%, 11.81% and 11.58% respectively (Yunus *et al.,* 2008; Uddin *et al.,* 2010; Razia *et al.,* 2012; Sultana *et al.,* 2012 and Rajkumar *et al.,* 2017). This mild variation in percent prevalence may be observed due to the geographical variation, higher prevalence of infection in a study area and various secondary factors that contributes to the increased virulence of organism to produce clinical disease. In the current study the significant lesions observed in birds suspected for CRD were similar to the earlier findings (Islam *et al.,* 2011). Haemorrhages in trachea were similar to the findings of Brar *et al.* (2017) and the thickened mucosa and infiltration of cells were in accordance with Stipkovits *et al.* (2012). Hypertrophy of mucous gland, mucosal thickening, infiltration of leukocytes and surface epithelial destruction were in accordance with histopathological findings in trachea (Thilagavathi *et al.,* 2016). Presence of congestion, haemorrhage and cell infiltration in lungs were in accordance to many authors' findings of CRD in poultry (Islam *et al.,* 2011; Brar *et al.,* 2017 and Thilagavathi *et al.,* 2017). In our study maximum of severe type of congestion (45.45%) was observed, which was in discordance with other study where moderate type of congestion (42.86%) was most prevalent in birds showing respiratory lesions (Itoo *et al.,* 2014). In another study the bronchopneumonia was observed in MG infection at 2.00% of the cases (Ibrahim *et al.,* 2015); the percent occurrence of bronchopneumonia in our current study was 69.50%. The occurrence of catarrhal and fibrinous type of bronchopneumonia in birds affected with MG has been reported (Yilmaz and Timurkaan, 2011). Timurkaan *et al.* (2008) found the most prevalent type of pneumonia observed in broilers were catarrhal type of bronchopneumonia. In our current study we found the percent occurrence of catarrhal type of bronchopneumonia as 19.44%. Secondary bronchial hyperplasia was noticed in 27.77% of birds affected with CRD. This finding was also in agreement with earlier reports where bronchial hyperplasia in birds affected with CRD has been reported (Sivaseelan *et al.,* 2013 and Thilagavathi *et al.,*

2017). In the current study the molecular diagnosis of MG was found to be 13.33%. In accordance with our findings Rajkumar *et al*. (2018) determined the prevalence of MG by PCR as 11.65%. A higher prevalence of 18.46% and 27.00% respectively was reported by Reddy (2014) and Tomar *et al*. (2017). The wide variation of MG prevalence and detection rates in different studies might be due to the sample size, sample type, detection techniques, rate of infection, biosafety and biosecurity in the respective study area (Rajkumar *et al.,* 2018). Sample in the present study were largely based on organised farms where strict biosecurity measures are practised. Good biosecurity management practices followed in farms may play a major role in control of organisms which in turn can be manifested as low detection of organisms from sampling. The lack of identification may occur due to low antigen concentrations in the samples, which is common for chronic infections like chronic respiratory disease (Lockaby *et al.,* 1998). The stage of infection may also influence the detection of organism as tracheal or choanal number of organisms in chronically infected birds may be so low that *Mycoplasma gallisepticum* organism may not be detected by sampling. In other words acute infections generally have higher DNA copies in samples than chronic infections, as in chronic infection attenuation of organism due to immune response takes place (Hossam *et al*., 2016). MG affects younger birds more severely than mature birds. In the present study 75.00% of birds found positive for *Mycoplasma gallisepticum* were less than three weeks of age and 25% of samples positive for *Mycoplasma gallisepticum* were from birds of seven to eight weeks of age. This finding was in accordance with the study where the prevalence of MG was found high in birds of 8 to 21 days of age (E. Haque *et al.,* 2015). This higher prevalence of *Mycoplasma gallisepticum* at broiler chicks may be due to vertical transmission in commercial breeder poultry flocks of India, which results in spread of MG infection from breeder hens to chicks (Tomar *et al.,* 2017). Yoder (1972) found development of resistance in MG infected adult birds. This development of immunity against infection may also decrease the detection of organism in adult birds. Thus higher infection in the young chickens might be due to the lesser immunity level and it can be speculated that, until and unless parent flocks are not free from mycoplasmosis, the disease cannot be controlled from commercial flocks. To conclude a significant prevalence of CRD was observed in the study area. *Mycoplasma gallisepticum* inducing destruction of cilia and tracheal epithelial cells could lead to immune modulations and superimposed infections. Strict biosafety measures are to be applied to reduce economic losses in the poultry industry.

REFERENCES

Bahatti, I., S.A. Rizvi, R. Sultana, Y.S. and Y.S. Mustafa, 2013. Prevalence and HI titer of Chronic Respiratory Disease (CRD) in broiler and layers commercial farms in District Lahore. *Science International*, Vol. 24, pp. 159-161.

Brar, R.S., G. D. Leishangthem, P. D. Gadhare, N. D. Singh, H. S. Banga, S. Sodhi, 2017. Histopathological and immunohistochemical studies on cases of chronic respiratory disease in poultry. *Indian Journal of Veterinary Pathology*, Vol. 41, pp. 232-234.

Gridley M. F. 1960. Manual of Histological and Special Staining Technique. McGraw-hill book company, New York, USA, p 202.

Haque E., G. Uddin, S. Akter, 2015. Prevalence of mycoplasmosis of chickens at Kotwali thana in Chittagong, *Bangladesh Journal of Fisheries and Livestock Production*, Vol. 3, p 151.

Hossam M., A. Wagih, E. Mona, E. Mahmoud, E. Rehab, E. T. Salah, 2016. The recovery and molecular diagnosis of *Mycoplasma gallisepticum* infection in commercial poultry flocks in Egypt. *Indian Journal of Science and Technology,* Vol. 9, pp. 1-8.

Ibrahim, Z. Y., B. H. Ali, N. M. Saeed and P. M. A. Rashid, 2015. Molecular and clinical study of caseaous bronchopneumonia in broilers. *Indian Journal of Research*, Vol. 4, pp. 1-3.

Islam A., A. Aslam, Z. I. Chaudhry, M. U. D. Ahmed, H. U. Rehman, K. Saeed and I. Ahmed, 2011. Pathology of *Mycoplasma gallisepticum* in naturally infected broilers and its diagnosis through polymerase chain reaction. *International Journal of Agriculture and Biology*, Vol.13, pp. 835-837.

Itoo F. A., M. S. Mir, S. A. Kamil, H. M. Khan and M. M. Darzi, 2014. Histopathological profiling of respiratory tract lesions in chickens. *SKUAST Journal of Research*, Vol. 16, pp. 93-104.

Ley D. H. 2008. *Mycoplasma gallisepticum* infection. In: Diseases of Poultry. (Y. M. Saif, Ed.), 12th ed., Wiley-Blackwell publishing, Oxford, England, pp. 722-744.

Lockaby S. B., F. J. Hoerr, L. H. Lauerman and S. H. Kleven, 1998. Pathogenicity of *Mycoplasma synoviae* in broiler chickens. *Veterinary Pathology*, Vol. 35, pp. 178-190.

Mcdonald J. H. 2014. Test of Nominable Variables. In: Handbook of Biological Statistics, 3rd ed., Sparky house publishing, Baltimore, Maryland, USA, 77p.

OIE, 2008. Manual of diagnostic tests and vaccines for terrestrial animals: Avian mycoplasmosis (*Mycoplasma gallisepticum* and *M. synoviae*), Office of International des Epizooties, Paris, France, pp. 482-496.

Rajkumar S., M. R. Reddy and R. Somvanshi, 2017. Incidence and risk factors of chronic respiratory disease in Indian poultry flocks. *International Journal of Science and Environmental Technology*, Vol. 6, pp. 662-668.

Rajkumar S., M. R. Reddy and R. Somvanshi, 2018. Molecular detection and pathology of spontaneous cases of chronic respiratory disease in chicken. *Indian Journal of Veterinary Pathology*, Vol. 41, pp. 277-282.

Ramadass, P., R. Ananthi, T. M. A. Senthilkumar, G. Venkatesh and G, V. Ramaswamy, 2006. Isolation and characterization of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* from poultry. Indian Journal of Animal Sciences, Vol. 76, pp. 796-798.

Razia S., S. Bushra, A. Rahat, S. Chaudhary and M. Azhar, 2012. A study on the prevalence of respiratory diseases in broiler and layer flocks in and around Lahore district. *Punjab University Jouirnal of Zoology*, Vol. 27, pp. 13-17.

Reddy M. R. 2014. Prevalence of *Mycoplasma gallisepticum* infection in Indian poultry farms. Proceedings of the Second International Conference on Animal and Dairy Sciences, 15-17th, September, Hyderabad, India.

Saritha N. S., B. M. Veeregowda, G. R. Reddy, D. Rathnamma, G. Leena, Y. L. V. Kumar, P. Sagar and C. S. Nagaraja, 2010. Isolation and Molecular characterization of *Mycoplasma synoviae* from chicken. *Indian Journal of Animal Sciences*, Vol. 80, pp. 497-499.

Sivaseelan S., S. Malmarugan, P. Balachandran and G. A. Balasubramaniam, 2013. Synergistic pathological effect of *Mycoplasma gallisepticum* with other infectious organisms

in layer chickens. *Brazilian Journal of.Veterinary Pathology*, Vol. 6, pp. 44-47.

Stipkovits L., L. Egyed, V. Palfi, A. Beres, E. Pitlik, M. Somogyi, S. Szathmary and B. Denes, 2012. Effect of low-pathogenicity influenza virus H3N8 infection on *Mycoplasma gallisepticum* infection of chickens. *Avian Pathology*, Vol. 41, pp. 51-57.

Sultana R., B. Siddique, R. Ali, S. Chaudhary and A. Maqbool, 2012. A study on the prevalence of respiratory diseases in broiler and layer flocks in and around Lahore district. *Punjab University Journal of Zoology*, Vol. 27, pp. 13-17.

Thilagavathi K., S. Sivaseelan, G. A. Balasubramaniam and A. Balasubramaniam, 2016. Pathology of *Mycoplasma gallisepticum* infection in naturally infected layer birds. *Indian Journal of Veterinary Pathology*, Vol. 40, pp. 337-340.

Thilagavathi K., S. Sivaseelan, G. A. Balasubramaniam, A. Balasubramaniam, A. Arulmozhi and R. Madheswaran, 2017. Detection of *Mycoplasma gallisepticum* from field samples of laying chicken using PCR. *International Journal of Science, Environment and Technology*, Vol. 6, pp. 2594-2599.

Timurkaan N., F. Yilmaz, A. Kilic and G. Ozbey, 2008. Pathological and microbiological investigations on lung lesions of slaughtered broilers in the slaughter house. *Journal of Animal and Veterinary Advances* Vol.7, pp. 1358-1363.

Tomar, P., Y. Singh, N. K. Mahajan, N. Jindal and M. Singh, 2017. Molecular detection of avain mycoplasmas in poultry affected with respiratory infections in Haryana, India. *International Journal of Current Microbiology and Applied Sciences*, Vol. 6, pp. 2155-2162.

Uddin M. B., S. S. U. Ahmed, M. M. Hassan, S. A. Khan and M. A. Mamun, 2010. Prevalence of poultry diseases at Narsingdi, Bangladesh. *International Journal of Biological Research*, Vol. 1, pp. 9-13.

Yilmaz F. and N. Timurkaan, 2011. Detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* antigens by immuno histochemical method in pneumonic broiler chicken lungs. *Journal of Animal and Veterinary Advances*, Vol. 10, pp. 2557-2560.

Yoder H. W. 1972. *Mycoplasma gallisepticum*. In: Diseases of Poultry, 6th ed., State University Press, Ames, Iowa, USA, pp. 287-307.

Yunus A. W., M. K. Nasir, U. Farooq and J. Bohm, 2008. Prevalence of poultry diseases and their interaction with mycotoxicosis in district Chakwal: Effects of age and flock size. *Journal of Animal and Plant Sciences,* Vol.18, pp. 107-113.

****