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Seroprevalence of *Mycoplasma gallisepticum* amongst Commercial Layers in District Peshawar, Khyber Pakhtunkhwa Pakistan

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ABSTRACT: In poultry *Mycoplasma gallisepticum* is an important chronic respiratory disease causing pathogen. In Pakistan the most harmful problem faced by poultry industry is Mycoplasmosis. *Mycoplasma gallisepticum* (MG) seroprevalence and role in cases of chronic respiratory disease (CRD) in commercial layer have not been reported in district Peshawar. Therefore this study was conducted with the objective to find out seroprevalence of *Mycoplasma gallisepiticum* in commercial layer chicken in some regions of Peshawar, Khyber Pakhtunkhwa Pakistan. A total of 500 blood samples were randomly obtained from 25 commercial layer flocks with respiratory issues. This study was conducted for a period of one year from January to December 2018. Serum plate agglutination test was used against *Mycoplasma gallisepticum* antigen by subjecting all the sera samples. +1 to +3 score was assigned for agglutination. All the samples having agglutination score +2 or greater were considered as positive. 12 flocks were observed having *Mycoplasma gallisepiticum* infection. This indicates an overall contribution of 48% amongst the respiratory problems. Amongst 12 positive flocks for *Mycoplasma gallisepiticum,* pullets was found to have highest seroprevalence of 55.55 %, in adult it was 46.15 % and 33.33 % was observed in old laying flocks. The infection due to *Mycoplasma gallisepiticum* was observed to have more seroprevalence in winter season (50 %) than summer season (40 %). Higher seroprevalence rate (55%) was documented in high bird density flocks than low density flock in which 20 % of the seroprevalence was observed. Our study concludes that *Mycoplasma gallisepticum* is highly prevalent in commercial layers in district Peshawar. In Pakistan this high seroprevalence of *Mycoplasma gallisepticum* stresses to frame national policy for regular monitoring and disease control. Therefore our study suggests that there should be routine monitoring of commercial layer farms for infection of *Mycoplasma gallisepticum.*

Keywords: Seroprevalence; Commercial layers; Mycoplasmosis; *Mycoplasma gallisepticum*

I. INTRODUCTION

During the last few decades, poultry industry has made notable development in Pakistan, starting from backyard to a noticeably erudite commercial industry but along with the development of poultry industry, certain infectious pathogens causes economic losses by causing different diseases. Poultry farming is a risky business due to inadequate actions and management implemented by the stakeholders to control the diseases. The major health issues to the poultry industry are caused by numerous viruses and bacteria. Amongst these pathogens mycoplasmosis is the most important problem to the poultry industry. Four main pathogens cause mycoplasmosis that includes *Mycoplasma gallisepticum*, *mycoplasma synoviae*, *Mycoplasma meleagrides* and *Mycoplasma iowae* [1]. In comparison to other species of *Mycoplasma*, the major cause of chronic respiratory disease is *Mycoplasma gallisepticum* and gives more economical losses [2]. The important role of the poultry industry is good quality animal protein production in low expenses. *Mycoplasmas* are considered as an important pathogen of poultry industry. In chickens and turkeys it causes chronic respiratory diseases and synovitis or bursitis resulting great loses to economy of the world poultry business [3]. Because of downgrading of carcasses at slaughter, infection due to *Mycoplasma gallisepticum* causes substantial economic losses in the poultry industry [4]. In chickens, turkeys and other bird species, reduction of egg production has been reported due to this infection [5]. This pathogen is found naturally in chickens and turkeys but in numerous wild birds and other domestic animals, natural occurring infections due to *Mycoplasma gallisepticum* has also been reported [6].

Approximately all the chickens are affected by this infection but however the severity and the duration of the infection is variable. It causes more severe infection

for long duration in winter and younger birds are more severely affected as compared to old birds [2]. In adult flocks tracheal rales, nasal discharge, and coughing are the characteristic signs of the naturally occurring disease [7]. Production of egg is reduced in laying flocks, but at a lower level it is usually sustained [8]. On the other hand, serologic evidence of infection in the flocks might be present with no clinical sign, particularly if they got infection at a younger age and get recovery [9]. Infection due to *Mycoplasma gallisepticum* has transmission route largely through ovaries [10]. In multi-age production farms, maintaining free *Mycoplasma gallisepticum* flock is difficult or in some cases may be impossible hence it will account for additional costs of control program and vaccination. The control programs like surveillance for *Mycoplasma gallisepticum* must be performed in breeder flocks [9]. For subclinical infection in the flock, the only reliable tools for detection are serological testing [11]. In Iran two main Serological testing methods were used for poultry farms screening. These two methods include Serum Plate Agglutination (SPA) and Enzyme Linked Immunosorbent Assay (ELISA). Difference in sensitivity and specificity was observed between these two methods [12]. In district Faisalabad, amongst 430 flock with respiratory problems mycoplasmosis incidence of 12.69% of was observed in a previous study [13]. From 1991 to 1995, in Faisalabad and northern areas of Pakistan seroprevalence of *Mycoplasma gallisepticum* was (35.8%) and *Mycoplasma synoviae* was (28.30 %) [14]. The patho-epidemiological investigation was carried out to know the incidence and mortality pattern in poultry affected with chronic respiratory disease in Gonda district of Uttar Pradesh, India. The overall incidence was 12.64% with mortality of 9.10% [24].

In a previous study done by Mukhtar *et al.,* reported 37.40 % of the seroprevalence of mycoplasmosis. He reported high seroprevalence of *Mycoplasma gallisepticum* (90.20%) as compared to *Mycoplasma synoviae* (15.70 %) [13]. In Pakistan the most harmful problem faced by poultry industry is mycoplasmosis. *Mycoplasma gallisepticum* (MG) seroprevalence and role in cases of chronic respiratory disease (CRD) in commercial layer have not been reported in district Peshawar, Khyber Pakhtunkhwa Pakistan. This study will help to "frame national policy for regular monitoring and disease control".

II. MATERIALS AND METHODS

This study was conducted after approval from research committee and Institutional animal ethics committee. A total of 500 blood samples were randomly obtained from 25 commercial layer flocks with respiratory issues. This study was conducted for a period of one year from January to December 2018.

For serological testing all the 500 blood samples were collected randomly. There was no vaccination history of the included commercial layer in our study. From wing vein of all birds, blood samples were collected aseptically in a 3 ml sterilized disposable plastic syringe having no anticoagulant. In the syringe the blood was allowed to clot for about one hour. For 4-5 hours syringes having blood samples were preserved at 4℃ in the refrigerator. The serum was transferred to the centrifuge tube. In order to get clear serum, the centrifuge tubes were centrifuged for five minute at 2500 rpm. In sterile Eppendorf tube, serum was collected and at -20℃. It was preserved for further processing. Serum plate agglutination test was used against *Mycoplasma gallisepticum* antigen by subjecting all the sera samples. +1 to +3 score was assigned for agglutination. All the samples having agglutination score +2 or greater were considered as positive [13]. All the data was analyzed statistically. Chi-square test was used for categorical variables by considering p value less than 0.05 as significant.

III. RESULTS

In our study a total of 500 blood samples were collected randomly from 25 commercial layer flocks having respiratory problems in district Peshawar. This study was conducted for a period of one year from January to December 2018 to determine seroprevalence of *Mycoplasma gallisepticum* amongst Commercial Layers in District Peshawar, Khyber Pakhtunkhwa Pakistan. For detection of *Mycoplasma gallisepiticum* in commercial layers in district Peshawar, serum plate agglutination (SPA) test was used. 12 flocks were observed to having *Mycoplasma gallisepiticum* infection. This indicates an overall contribution of 48% amongst the respiratory problems (Table 1). Amongst 12 positive flocks for *Mycoplasma gallisepiticum,* pullets was found to have highest seroprevalence of 55.55 %, in adult it was 46.15 % and 33.33 % was observed in old laying flocks (Table 3 and Fig. 5). The infection due to *Mycoplasma gallisepiticum* was observed to have more seroprevalence in winter season (50 %) as compared to summer season (40 %) (Table 4 and Fig. 6). Higher seroprevalence rate (55%) was documented in high bird density flocks than low density flock in which 20 % of the seroprevalence was observed (Table 5 and Fig. 7).

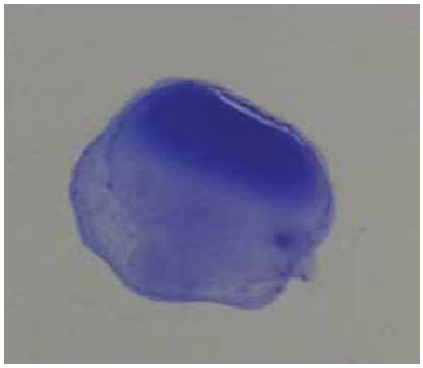
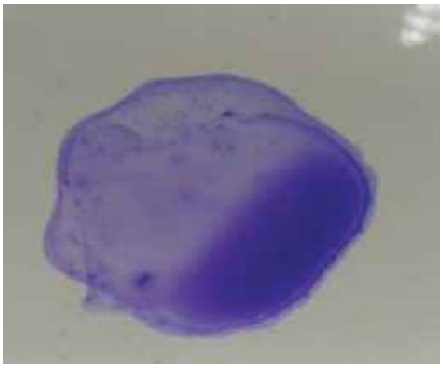


Fig. 1. + by SPA test.



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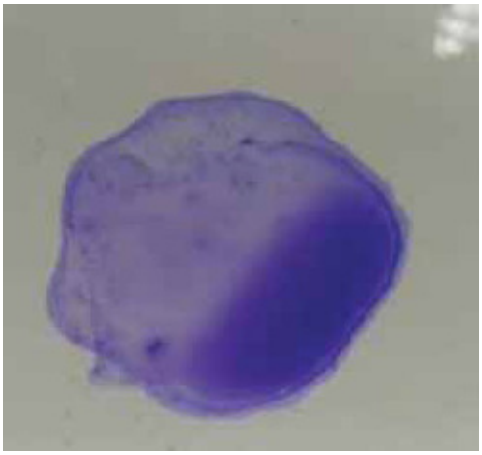

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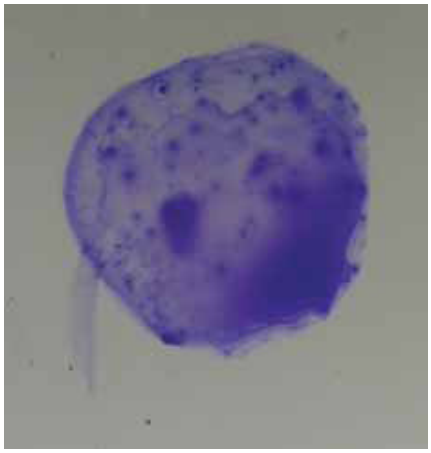

Fig. 4. ++++ by SPA test.

Table 1: Over all seroprevalence of *Mycoplasma gallisepiticum* in commercial layers on the basis of flocks.

Bird type	Total flocks	Positive n (%)	Negative n (%)
Commercial layer	25	12 (48)	13 (52)

Table 2: Over all seroprevalence of *Mycoplasma gallisepiticum* in commercial layers on the basis of no of samples.

Bird type	Total sample	Positive n (%)	Negative n (%)
Commercial layer	500	228 (45.6)	272 (54.4)

Table 3: Seroprevalence of *Mycoplasma gallisepiticum* in commercial layers among various age group flocks.

Age group	No of flocks	Positive n (%)	Negative n (%)	P value
Pullets	9	5 (55.55)	4 (44.44)	0.786
Adults	13	6 (46.15)	7 (53.84)	
Olds	3	1(33.33)	2 (66.66)	
Total	25	12 (48)	13 (52)	

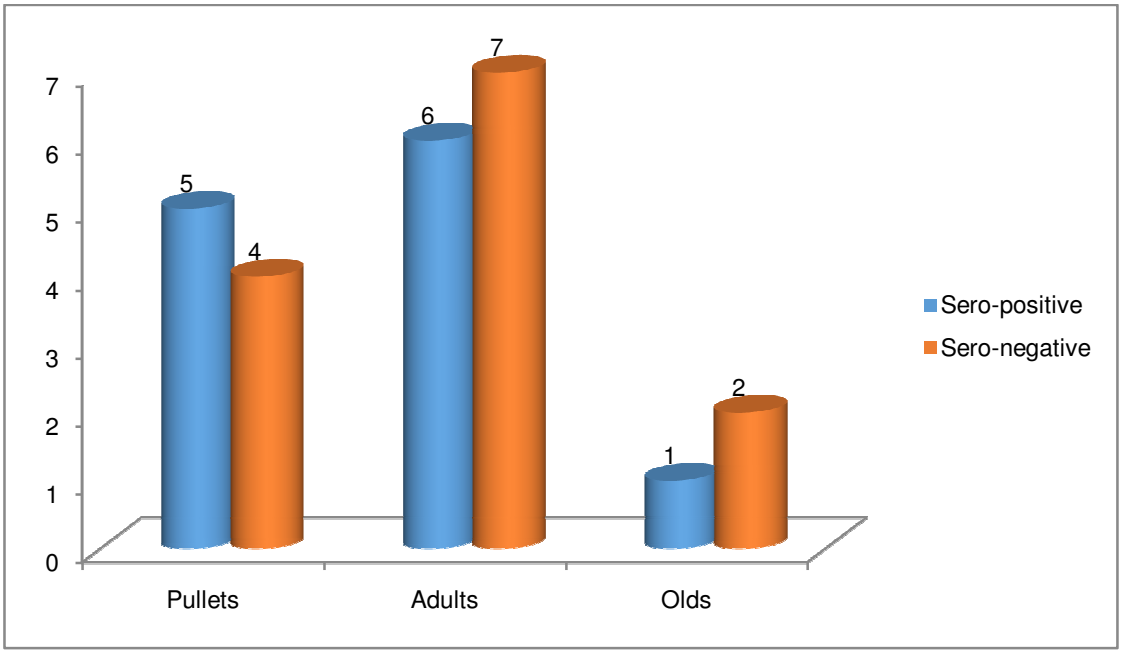

Fig. 5. Seroprevalence of *Mycoplasma gallisepiticum* in commercial layers among various age group flocks.

Table 4: Seasonal Seroprevalence of *Mycoplasma gallisepiticum* in commercial layers among various flocks.

Season	No of flocks	No of blood samples taken	Positive n (%)	Negative n (%)	P value
Winter	14	280	140(50)	140(50)	0.026*
Summer	11	220	88 (40)	132 (60)	
Total	25	500	228 (45.6)	272 (54.4)	

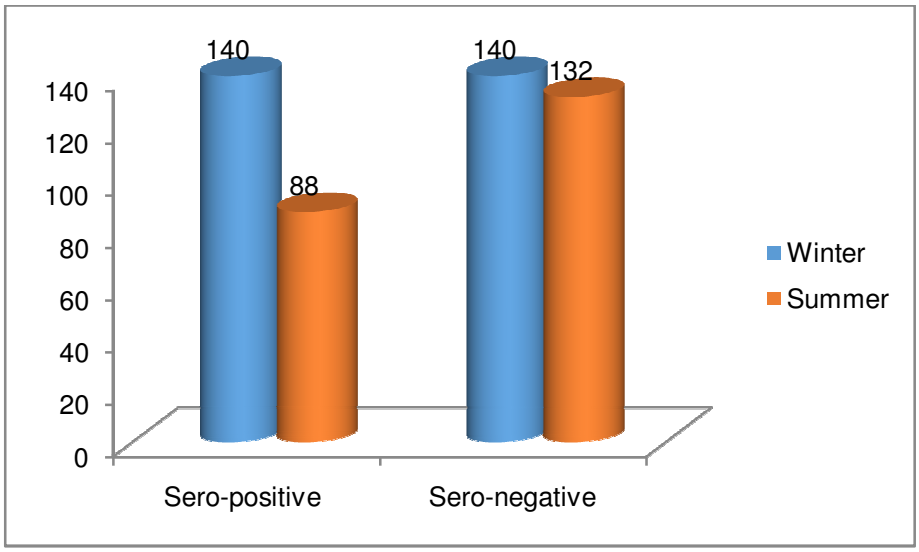

Fig. 6. Seasonal Seroprevalence of *Mycoplasma gallisepiticum* in commercial layers among various flocks.

Table 5: Seroprevalence of *Mycoplasma gallisepiticum* in commercial layer based on density of flocks.

Flock density	Number of flocks	Number of blood samples	Positive n (%)	Negative n (%)	P value
500-1000	2	40	8 (20)	32 (80)	<0.001*
1001-2000	2	40	12 (30)	28 (70)	
2001-3000	3	60	21(35)	39(65)	
3001-4000	3	60	27 (45)	33 (55)	
4001-5000	5	100	50 (50)	50 (50)	
5001 plus	10	200	110(55)	90 (45)	
total	25	500	228 (45.6)	272 (54.4)	

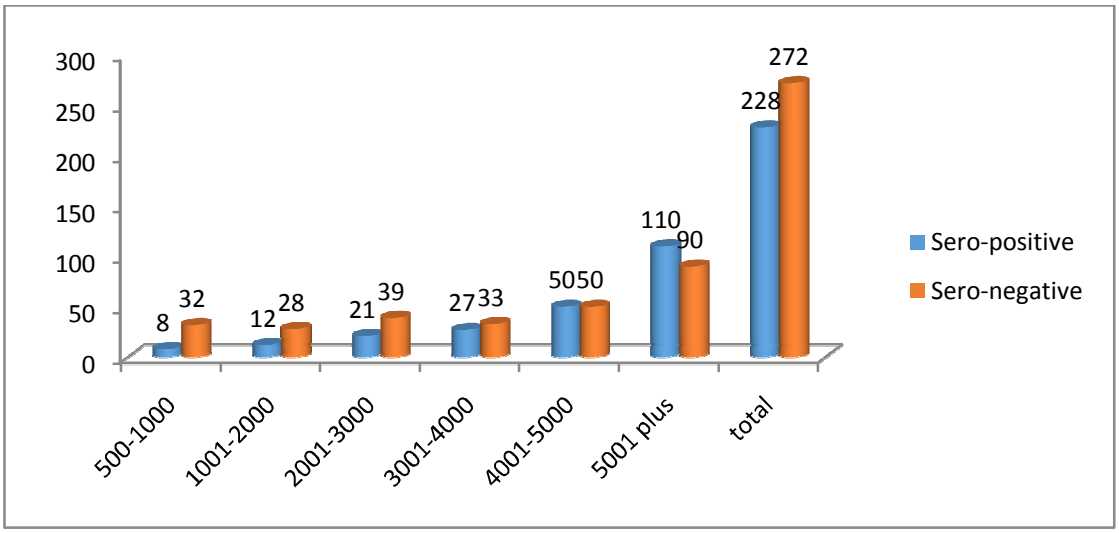

Fig. 7. Seroprevalence of *Mycoplasma gallisepiticum* in commercial layer based on density of flocks.

With respect to age, no significant association (p=0.786) was observed with seroprevalence of *Mycoplasma gallisepticum* while significant association of the *Mycoplasma gallisepticum* was observed with the season (p=0.026) and density of the flock (p=<0.001).

IV. DISCUSSION

In poultry *Mycoplasma gallisepticum* is an important chronic respiratory disease causing pathogen. This pathogen is found naturally in chickens and turkeys but in numerous wild birds and other domestic animals, natural occurring infections due to *Mycoplasma gallisepticum* has also been reported [6]. In our study a total of 500 blood samples were collected randomly from 25 commercial layer flocks having respiratory problems in district Peshawar. This study was conducted for a period of one year from January to December 2018 to determine seroprevalence of *Mycoplasma gallisepticum* amongst Commercial Layers in District Peshawar, Khyber Pakhtunkhwa Pakistan. For detection of *Mycoplasma gallisepiticum* in commercial layers in district Peshawar, serum plate agglutination (SPA) test was used. 12 flocks were observed to having *Mycoplasma gallisepiticum* infection. This indicates an overall contribution of 48% amongst the respiratory problems. Amongst 12 positive flocks for *Mycoplasma gallisepiticum,* pullets was found to have highest seroprevalence of 55.55 %, in adult it was 46.15 % and 33.33 % was observed in old laying flocks. The infection due to *Mycoplasma gallisepiticum* was observed to have more seroprevalence in winter season (50 %) as compared to summer season (40 %). Higher seroprevalence rate (55%) was recorded in flocks having high bird density as compared to low density flock in which 20 % of the seroprevalence was observed. Overall seroprevalence of *Mycoplasma gallisepticum* in our study was observed as 48%. Our result shows higher seroprevalence as compared to a previous reported study in Faisalabad city of Pakistan who reported a seroprevalence of 43.80% [15]. Our finding is in accordance with the previous reported studies [16-18] who observed seroprevalence of *Mycoplasma gallisepticum* as 54.90%, 52% and 53% respectively. Correspondingly, in Feni district of Bangladesh, Sarkar and his colleagues reported 58.9% seroprevalence of *Mycoplasma gallisepticum* in layers [19].

Additionally, in laying hens of Rajshahi and Batna districts of Estern Algeria and its surroundings, Hossain [20], Heleili [21] and his colleagues reported high seroprevalence of *Mycoplasma gallisepticum* as 45.10% and 81.15 % respectively. In early study done in Faisalabad city of Pakistan by Mukhtar reported the seroprevalence of *Mycoplasma gallisepticum* as 31.60%, 17.90%, 11.20% and 10.00% in pullets, chicks, adults and old layer respectively [13].

Correspondingly, an earlier study reported high seroprevalence of *Mycoplasma gallisepticum* infection (71.42%) in 18 weeks of age as compared to 63 weeks of age in which comparatively low seroprevalence was observed (55.17%) in layers [22]. In our study the infection due to *Mycoplasma gallisepiticum* was observed to have more seroprevalence in winter season (50 %) as compared to summer season (40 %). Similar results were reported in an earlier study, in which the seroprevalence of *Mycoplasma gallisepticum* infection in winter and summer was (61.48 %) and (47.74 %) respectively [23]. In our study Higher seroprevalence rate (55%) was documented in high bird density flocks than low density flock in which 20 % of the seroprevalence was observed. Our results are in accordance with the study done by Hossain *et al.* who reported high seroprevalence (51.4 %) of *Mycoplasma gallisepticum* in large flocks as compared to small flocks in which comparatively lower seroprevalence (41.3 %) [20].

V. CONCLUSION

Our study concludes that *Mycoplasma gallisepticum* is highly prevalent in commercial layers in district Peshawar. With respect to age, no significant association was observed with seroprevalence of *Mycoplasma gallisepticum* while significant association of the *Mycoplasma gallisepticum* was observed with the season and density of the flock. In regard to our findings, there is a need of strong effort to educate the farmers about this hazardous problem. Furthermore, biosecurity in commercial layer farms should also be enhanced. In Pakistan this high seroprevalence of *Mycoplasma gallisepticum* stresses to frame national policy for regular monitoring and disease control. Therefore our study suggests that there should be routine monitoring of commercial layer farms for infection of *Mycoplasma gallisepticum.*

FUTURE SCOPE

This study will help to “frame national policy for regular monitoring and disease control”.

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Conflict of interest. There exists no conflict of interest.

Author contributions. All authors contributed equally.

REFERENCES

[1]. Ahmad, A., Rabbani, M., Yaqoob, T., Ahmad, A., Shabbir, M., and Akhtar, F. (2008). Status of IgG antibodies against *Mycoplasma gallisepticum* in non-vaccinated commercial poultry breeder flocks. *J. Poult. Sci*. *18*(2-3): p. 61-63.

[2]. Ley, D.H. and Yoder Jr, H. (2008). *Mycoplasma gallisepticum* infection. *Diseases of poultry*. *12*: p. 807-834.

[3]. Beylefeld, A., Wambulawaye, P., Bwala, D.G., Gouws, J.J., Lukhele, O.M., Wandrag, D.B.R., and Abolnik, C. (2018). Evidence for multidrug resistance in nonpathogenic *Mycoplasma* species isolated from South African poultry. *Applied environmental microbiology*. *84*(21).

[4]. Adell, E., Calvet, S., Pérez-Bonilla, A., Jiménez-Belenguer, A., García, J., Herrera, J., and Cambra-Lopez, M. (2015). Air disinfection in laying hen houses: Effect on airborne microorganisms with focus on *Mycoplasma gallisepticum. Biosystems Engineering*. *129*: p. 315-323.

[5]. Ley, D., *Mycoplasma gallisepticum Infection (Eds. Saif, YM) Disease of Poultry*. 2008, Wiley-Blackwell Publishing, Iowa, IA.

[6]. Dhama, K., Mahendran, M., and Tomar, S. (2008). Pathogens transmitted by migratory birds: threat perceptions to poultry health and production. *International Journal of Poultry Science*. *7*(6): p. 516-525.

[7]. Ahmad, A., Rabbani, M., Yaqoob, T., Ahmad, A., Shabbir, M., and Akhtar, F. (2008). Status of IgG antibodies

against *Mycoplasma gallisepticum* in non-vaccinated commercial poultry breeder flocks. *Int. J. Poult. Sci*. *18*(2-3): p. 61-63.
[8]. Roussan, D.A., Khawaldeh, G., and Shaheen, I.A. (2015). A survey of *Mycoplasma gallisepticum* and *Mycoplasma synovaie* with avian influenza H9 subtype in meat-type chicken in Jordan between 2011–2015. *Poultry Science*. *94*(7): p. 1499-1503.
[9]. Jalilnia, M. and Movassagh, M.H. (2011). A study on causes of poultry carcasses condemnation in East Azerbaijan province (North West of Iran) poultry slaughterhouse. *Annals of Biological Research*. *2*(4): p. 343-347.
[10]. Wang, J., Ishfaq, M., Fan, Q., Chen, C., and Li, J. (2020). A respiratory commensal bacterium acts as a risk factor for *Mycoplasma gallisepticum* infection in chickens. *Veterinary Immunology and Immunopathology*. *230*: p. 110127.
[11]. Yadav, J.P., Tomar, P., Singh, Y., and Khurana, S.K. (2021). Insights on *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infection in poultry: a systematic review. *Animal Biotechnology*: p. 1-10.
[12]. Udhayavel, S., Gopala, T.R., Murthy, K., Gowthaman, V., and Senthilvel, K. (2018). Detection of seroprevalence of *Mycoplasma gallisepticum* in broiler chicken in Tamil Nadu by ELISA.
[13]. Mukhtar, M., Awais, M.M., Anwar, M.I., Hussain, Z., Bhatti, N., and Ali, S. (2012). Seroprevalence of Mycoplasma gallisepticum among commercial layers in Faisalabad, Pakistan. *Journal of Basic Applied Sciences*. *8*(1).
[14]. Ahmad, I., *Diagnosis and control of Mycoplasma gallisepticum infection in poultry*. 1998, Final Technical Report, Poultry Research Institute, Rawalpindi, Pakistan.
[15]. Godoy, A., Andrade, L., Colmenares, O., Bermudez, V., Herrera, A., and Munoz, N. (2001). Prevalence of M. gallisepticum in egg-laying hens. *Vet. Trop*. *26*(1): p. 25-33.
[16]. Biswas, P., Rahman, M., Biswas, D., and Ahmed, S. *A longitudinal study on the prevalence of endemic diseases affecting semi-scavenging poultry reared under PLDP area*. in *ninth BSVER Conference, Publication*. 2003.
[17]. Pradhan, M., *Studies on avian mycoplasmosis: Prevalence, isolation, characterization and antigenic properties*. 2002, PhD Thesis Submitted to the Dept. of Microbiology and Hygiene, Faculty of ….
[18]. Dulali, R., *Seroprevalence and pathology of mycoplasmosis in sonali chickens*. 2003, MS Thesis, Department of Pathology, Faculty of Veterinary Science ….
[19]. Sarkar, S., Rahman, M., Rahman, M., Amin, K., Khan, M., and Rahman, M. (2005). Sero-prevalence of Mycoplasma gallisepticum infection of chickens in model breeder poultry farms of Bangladesh. *International Journal of Poultry Science*. *4*(1): p. 32-35.
[20]. Hossain, K.M.M., Hossain, M.T., and Yamato, I. (2010). Seroprevalence of *Salmonella* and *Mycoplasma gallisepticum* infection in chickens in Rajshahi and surrounding districts of Bangladesh. *International Journal of Biology*.
[21]. Heleili, N., Mamache, B., and Chelihi, A. (2011). Incidence of avian mycoplasmosis in the région of Batna, Eastern Algeria. *Veterinary World*. *4*(3): p. 101.
[22]. Sikder, A., Islam, M., Rahman, M.M., and Rahman, M. (2005). Seroprevalence of Salmonella and *Mycoplasma gallisepticum* infection in the six model breeder poultry farms at Patuakhali district in Bangladesh. *International Journal of Poultry Science*. *4*(11): p. 905-910.
[23]. Hossain, K., Ali, M., and Haque, M. (2007). Seroprevalence of *Mycoplasma gallisepticum* infection in chicken in the greater Rajshahi district of Bangladesh. *Bangladesh Journal of Veterinary Medicine*: p. 9-14.
[24]. Gupta, R.K., Niyogi, D., Joshi, N., Joshi, R.K., Singh, J., Mishra, A., Yadav, A.K. and Singh, D.D. (2021). Patho-epidemiological Study of Chronic Respiratory Disease of Poultry in Gonda District of Uttar Pradesh. *Biological Forum – An International Journal*, **13**(1): 191-195.

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