The Journal of Animal & Plant Sciences, 24(2): 2014, Page: 385-391
ISSN: 1018-7081

DETECTION OF *MYCOPLASMA GALLISEPTICUM* IN EXPERIMENTALLY INOCULATED LAYER BIRDS BY IMMUNOHISTOCHEMISTRY

[*]M. Rauf, [*]Z. I. Chaudhary, [**]M. Younas, [***]A. A. Anjum, [****]H. M. Khan and [***]M. A. Ali

[*]Department of Pathobiology, Baha-ud-Din Zakariya University, Multan, Pakistan
[**]Department of Pathology, College of Veterinary and Animal Sciences, Jhang.
[***] Department of Microbiology, University of Veterinary and Animal Sciences (UVAS), Lahore-54000, Pakistan.
[****] Department of Pharmacy, Lahore College for Women University, Jail Road, Lahore-54000, Pakistan.
Corresponding Author E-mail: aftab.anjum@uvas.edu.pk

ABSTRACT

Mycoplasma gallisepticum (MG), causative agent of chronic respiratory disease (CRD), is transmitted by both vertical and horizontal routes. Aim of the present study was to analyze the focal infection by immunohistochemistry. Two hundred (200) white leg horn layer birds, divided in four groups (n=50, each) were experimentally infected with MG by aerosol, intra-tracheal or oral routes respectively in relation to control group. Birds were monitored daily for the appearance of clinical signs of disease. Ten birds from each group were randomly selected and slaughtered on 4th, 11th, 18th, 25th and 32nd days post infection. Gross lesions including air sacculitis, salpingitis, putrefied eggs in ovary, and hemorrhages in trachea and lungs were detected. Histopathology of trachea, lungs and oviduct showed early onset of MG infection by aerosol, followed by intra tracheal and oral route. The infection through aerosol and intra-tracheal route was found to be severe as compare to oral route. Immunohistochemical examination of paraffin embedded sections of trachea, lungs and oviduct revealed the presence of MG in lungs and oviduct. Out of 150 MG infected birds 44 (29%) were positive on immunohistochemical examination. Highest percent infection was observed by intra-tracheal route (21/50) followed by aerosol (16/50) and per-oral (7/50). Result insinuates the importance of immunohistochemistry and relation between route of infection and disease severity of MG in birds.

Keywords: *Mycoplasma gallisepticum*, CRD, gross lesions, histopathology and Immunohistochemistry

INTRODUCTION

In Pakistan, poultry industry is comprised of rural and commercial rearing systems. There are more than 76 million rural poultry birds which contribute 32% of the egg production and 15% to the poultry meat production in the country. Poultry industry is facing a number of infectious and non infectious problems. Varieties of infections including bacteria, protozoa, fungi and virus result in devastating losses. Among bacterial infections chronic respiratory disease (CRD) caused by *Mycoplasma gallisepticum* (MG) is a serious threat to industry (OIE, 2008). MG infections cause significant economic losses to poultry industry in the form of reduced feed efficiency, hampered growth and lowered egg production. MG poultry infections are considered among top ranking infectious maladies word wide (OIE, 2008). This organism has been eradicated from most commercial chicken and turkey breeding flocks in the United States; however, it is endemic in many countries.

MG is the smallest, wall less, self-replicating prokaryote (Rottem, 2003) transmitted from infected to healthy birds both by horizontal and vertical routes (Glisson and Kleven, 1984; Bradbury, 1998). Sources of microbial spread include infected/healthy carrier birds, hatchery, housing, equipments, feeding, trafficking, fomites and vectors (Ley, 2003). Infected birds remain carriers for extended periods of time.

Control of MG could however, be possible through better health management, bio-safety, proper vaccination, and monitoring of flocks (Ahmad *et al*., 2008). Early and accurate diagnosis of infection helps to maintain MG free flocks. Vaccination is the only choice in situations where field exposure is inevitable. However, the disease is mostly transmitted from infected or carrier birds to healthy chicks. Thus, it is imperative for the farmers to have control measures and eliminate infected or carrier birds from the flocks.

Proper diagnosis and stage of disease is essential in order to design effective strategies for timely treatment and eradication of disease (Bencina and Dorrer, 1984). Different techniques are applied to diagnose MG in infected birds. Clinical signs and necropsy findings are usually confusing with many other respiratory disorders. Histopathology is a good tool in consultation with highly expert opinion. Highly sensitive serological and molecular techniques are being practiced for accurate detection of MG in poultry breeder flocks. Immunohistochemistry has proved to be excellent immunological technique for effective identification of MG antigens in predilection tissues for focal infection (Nunoya *et al*., 1997). Present plan was to assess the post infection lodging of MG in different tissues in relation to

time under experimental conditions by immunohistochemistry.

MATERIALS AND METHODS

Mycoplasma gallisepticum (MG) free layer birds (n=200) were reared under controlled conditions dividing in four groups A, B, C and D (n=50 each). Group A was kept as control, while the replicates of groups B, C and D were infected with field isolate of MG. Isolates declared as MG by growth inhibition test (Khalifa *et al*., 2008) were confirmed by polymerase chain reaction (Sankar *et al*., 2012). Birds of groups B, C and D were infected with 2.86×10^{10} CFU/ml MG culture by aerosol, intra-tracheal and oral routes, respectively.

Birds in all groups, monitored daily and clinical signs were recorded. Ten birds from each group were randomly selected and slaughtered on 4^{th}, 11^{th}, 18^{th}, 25^{th} and 32^{nd} day post infection. The birds incised and lesions observed in viscera were recorded. Tissue samples (trachea, lungs, liver and oviduct) collected from each experimental unit were preserved in 10 % buffered formalin. The histopathological examination was carried out on all samples according to standard procedure described by Bancroft and Gamble (2002). The slides were stained by Hemtoxylin and Eosin staining described by Bancroft and Gamble (2002). Histopathological changes in tissue were observed under bright field compound microscope (Olympia) at magnification of 100X and 400X and photographed.

MG antigen was detected in tissue using avidin biotin protocol provided by Nunoya *et al*. (1997). Steps performed in tissue processing and slide preparation till removal of xylene were same as for histopathology. The excessive quantity of absolute alcohol was removed by placing slides in 100%, 90% and 70% alcohol for three minutes each. Slides were dipped in jar containing water for 5 minutes and then in 3% H_2O_2 dissolved in 70% methanol for 10 minutes. Antigen was retrieved by digestion with Trypsin having concentration of 0.01% in PBS for 30 minutes at 37^0C. Blocking was performed by incubation in normal goat serum for 30 minutes at 4^0C. Tissue sections were incubated with primary antibodies against MG (raised in rabbit) at dilution of 1:800 in Tris-buffered saline tween-20 (NaCl 8.8g, KCl 0.2g, Tris base 3g, Tween-20 500ul and Distilled water up to 1L) with 2.5% bovine serum albumin (BSA) for overnight. Slides were rinsed and washed three times in PBS. Biotin labeled goat anti-rabbit immunoglobulins (1:300) in PBS with 2.5% BSA were applied as secondary antibody for an hour. Slides were rinsed and washed three times in PBS. The sections were incubated with 3-amino-9-ethylcarbazol H_2O_2 staining solution for 20 minutes. Slides were rinsed and washed three times in PBS and dipped in Hematoxylin stain for 1-2 minute. Slides washed with running tap water for 5 min and 2-4 quick dips were given in 70% acid alcohol. Slides were dipped for 5 minute in ammonia water. DPX and cover slip placed on it to convert in permanent mount. The slides were placed under microscope and examined for detailed immuno histochemical findings at 100 and 400X magnifications. Representative microscopic fields of tissue were photographed.

Data obtained was statistically analyzed at ($P \leq 0.05$) through SAS 9.1.3 portable software by using chi square test.

RESULTS

Clinical signs: All the birds in control group were normal in relation to feeding, drinking and egg production. No apparent clinical and pathological changes were observed in any bird. The birds of group B were slightly anorexic for 24 hour post infection (PI). The birds were depressed with ruffled feathers which may be due to stress and inflammatory response of body to the inoculums. Birds of this group were normal on day 6^{th} some birds showed signs of anorexia with mild respiratory signs. On day 10^{th} PI most of the birds showed signs of respiratory distress with conjunctivitis and nasal discharges. The signs were severe on day 15^{th} PI and clinical signs were slightly reduced in term of severity on day 19^{th} (PI). On day 23^{rd} and onward the birds were apparently normal with slight ruffled feathers. Birds of group C exhibited similar pattern of disease as described in Group B but signs were slightly earlier (day 5^{th} PI) and developed rapidly as compared to group B. Birds in group D were normal in first 24 hours and did not showed any apparent abnormal clinical behavior as compared to group B and C. Signs developed were very mild in nature and late (day 14^{th} PI) during course of study (table 1).

Gross lesions: No apparent gross lesions were noticed in any visceral organ of group A (control) birds till the end of study. On postmortem examination of group B, mild hemorrhages were observed in trachea at 4^{th} DPI. On clinical scoring, gross lesions were moderate at 11^{th} DPI with mild exudates in lumen of trachea, severe at 18^{th} and 25^{th} DPI and became moderate till 32^{nd} DPI. Mucous was serous at the start, became thick and purulent at advanced stage of disease. Cloudy exudates were found in the internal nostrils and conjunctiva sac. Air sacs were normal in all birds at 4^{th} DPI, but became thick cloudy at 11^{th} day. On 18^{th} DPI apparent and classical thick cloudy changes were observed in air sacs. The lesions remained consistent at 25^{th} and 32^{nd} DPI. On clinical scoring the gross lesions adopted specific pattern mild at the beginning became severe at 18^{th} day, chronic/persistent at 25^{th} DPI. The exudates turned into thick and cheesy in air sacs. Lungs were consolidated, hemorrhagic and frothy exudates came out from cut surface. Lesions were mild at

18th DPI, became moderate at 25th day and remained persistent up to 32nd DPI (table 2). Hemorrhages were observed in the lumen of oviduct with swollen lymph plexus. Lesions observed on 25th DPI were moderate in nature and remained persistent then became severe on 32nd DPI. The lesions in oviduct were more severe in nature. Putrefied egg yolk was found in ovary of six birds. All other visceral organs were almost normal. Pattern of postmortem lesions observed was in birds of group C was comparable with group B. Lesions developed very slowly and only few birds showed signs of disease in group D. Lesions appeared at 11th DPI in trachea, very mild in only few birds and became moderate on 32nd DPI (table 2).

Table 1. Comparative clinical scores in *Mycoplasma gallisepticum* experimental infection of birds by different routes in relation to time

Group	Intra tracheal Post infection Days					Aerosol Post infection Days					Per oral Post infection Days				
Clinical signs	**4**	**11**	**18**	**25**	**32**	**4**	**11**	**18**	**25**	**32**	**4**	**11**	**18**	**25**	**32**
Respiratory sounds	0	2	2	2	0	0	2	2	2	0	0	0	0	2	2
Open mouth breathing	0	2	2	2	0	0	2	2	2	2	0	0	2	2	2
Coughing/ Sneezing	0	2	2	2	2	0	2	2	2	2	0	0	0	2	2
Nasal discharge	0	2	2	0	0	0	2	2	2	2	0	0	2	2	2
Ruffled feathers	0	0	2	2	0	0	0	2	2	0	0	0	0	2	0
Overall condition	G	P	P	P	F	G	P	P	P	P	G	G	F	P	P

0= absent, 2= present, G=good, F= fair and P= poor

Table 2: Comparative gross lesion scores in *Mycoplasma gallisepticum* experimental infection of birds by different routes in relation to time

Organs	Group	Intra tracheal Post infection Days					Aerosol Post infection Days					Per oral Post infection Days				
	Postmortem signs	**4**	**11**	**18**	**25**	**32**	**4**	**11**	**18**	**25**	**32**	**4**	**11**	**18**	**25**	**32**
Trachea	Hemorrhages	1	2	3	3	2	1	3	3	3	3	0	1	1	1	2
	Exudates	0	1	2	2	1	1	2	2	2	2	0	0	0	1	1
	Total	1	3	5	5	3	2	5	5	5	5		1	1	2	3
Air sac	Thick	0	1	3	3	3	1	2	3	3	3	0	1	1	2	3
	Cloudy	0	1	2	3	3	0	2	3	3	3	0	0	0	0	1
	Exudates	0	0	0	1	1	0	0	1	1	2	0	0	0	0	0
	Total		2	5	7	7	1	4	7	7	8		1	1	2	4
Lungs	Hemorrhages	0	0	1	2	2	0	1	2	2	2	0	0	0	1	1
	Consolidation	0	0	0	1	1	0	0	1	1	1	0	0	0	0	0
	Total			1	3	3	0		3	3	3				1	1
Oviduct	Hemorrhages	0	0	0	2	3	0	0	1	3	3	0	0	0	1	1

0= absent, 1= mild, 2= moderate and 3= severe

Histopathology: The birds in control group were normal and no apparent histopathological lesions were noticed in any visceral organ throughout the study. In group B, at 4th DPI histological lesions observed were mild tracheal hemorrhages, leukocyte infiltration, thickness of membrane and cilia covered with layer of mucous. Lesions were severe on 11th DPI, accompanied hypertrophy of mucous glands and remained consistent till 25th day becoming slightly moderate on 32nd day (table 3). No significant changes were observed in lungs at early stages however, edema and mild hemorrhages were on 18th DPI. On 25th and 32nd DPI, histological lesions observed were edema, congestion, hemorrhages with extensive infiltration of polymorph nucleated cells, focal necrosis and fibrinous tissue with leukocyte infiltration. Liver was normal with no changes but mild congestion and leukocyte infiltration in spaces around portal vessel were observed at 32nd DPI. First sign of mild edema with leukocyte infiltration was observed at 18th DPI in ovary and converted to moderate followed by severe hemorrhages, leukocyte infiltration and sloughing of epithelium at 32nd DPI. On histopathology of group C lesions recorded were almost similar in nature as group B but were slightly severe and earlier in course of infection. In group D lesions developed very slowly as compared to group B and C. At 4th day post inoculation there was no apparent change and at 11th day there were mild hemorrhages in trachea. At 18th DPI, there was mild congestion of lungs and no significant sign in liver and oviduct observed at 25th and 32nd days (table 3).

Immunohistochemistry: None of the birds from control group revealed positive results. Overall 29 percent birds were declared positive by immunohistochemical technique among infected groups. Highest focal infection (10.5%) observed was by intraocular route followed by aerosol (8%) and oral (3.5%) routes, respectively. The positive immunohistochemical reaction was found as red colored spots at the reaction site. MG antigen was not detected in sloughed off epithelial cells of trachea. Focal infection was revealed in lungs particularly in inter-alveolar spaces, cytoplasm of macrophages and inter air-vesicle septa infiltrated with polymorph nucleated cells (table 4).

Table 3: Comparative histological lesion scores in *Mycoplasma gallisepticum* experimental infection of birds by different routes in relation to time

Group		Intra tracheal Post infection Days					Aerosol Post infection Days					Per oral Post infection Days				
Histopathology		**4**	**11**	**18**	**25**	**32**	**4**	**11**	**18**	**25**	**32**	**4**	**11**	**18**	**25**	**32**
Trachea	**L.I**	1	3	3	2	2	1	3	3	3	3	0	0	1	2	1
	M.T	1	2	2	2	3	1	2	3	3	3	0	0	1	2	2
	He	1	2	3	3	2	1	2	2	3	2	0	0	0	1	2
	H.M.G	0	1	2	3	2	0	1	2	2	2	0	0	0	1	2
	Total	3	8	10	10	9	3	8	10	11	10	0	0	2	6	7
Lungs	**C**	0	0	1	2	2	0	1	2	3	3	0	0	0	2	2
	L.I	0	0	1	1	2	0	2	2	1	2	0	0	0	1	2
	Total	0	0	2	3	4	0	3	4	4	5	0	0	0	3	4
Liver	**C**	0	0	0	0	1	0	0	1	1	0	0	0	0	0	1
	L.I	0	0	0	0	1	0	0	1	2	2	0	0	0	0	0
	Total	0	0	0	0	2	0	0	2	3	2	0	0	0	0	1
Oviduct	**L.I**	0	0	1	2	2	0	0	2	3	2	0	0	0	0	1
	E	0	0	1	1	2	0	0	1	1	2	0	0	0	0	1
	N	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
	Total	0	0	2	3	4	0	0	3	5	5	0	0	0	0	2

0= absent, 1= mild, 2= moderate, 3= severe; Leukocytes infiltration = L.I, Membrane thickness = M.T, Hemorrhages = He, Hyperplasia of mucous gland = H.M.G, Congestion = C, Edema = E, Necrosis = N

Table 4: Immunohistochemical analysis of *Mycoplasma gallisepticum* focal infection in viscera of birds by different routes in relation to time

Group	Intra tracheal Post infection Days					Aerosol Post infection Days					Per oral Post infection Days				
Postmortem signs	**4**	**11**	**18**	**25**	**32**	**4**	**11**	**18**	**25**	**32**	**4**	**11**	**18**	**25**	**32**
Trachea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lungs	0	0	2	5	8	0	1	3	7	8	0	0	1	3	3
Oviduct	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0

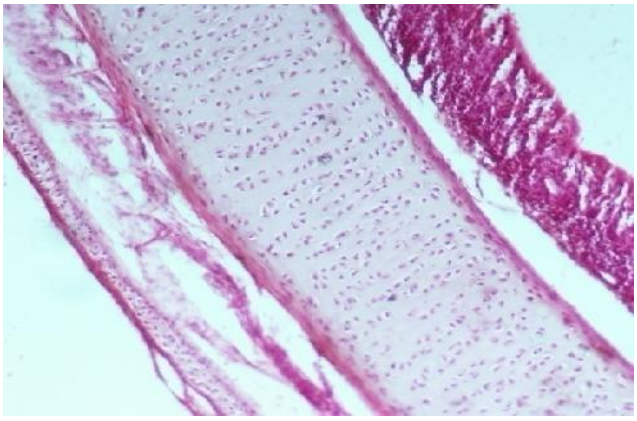

Plate 1: Histological lesions in trachea of MG infected bird at 100X magnification

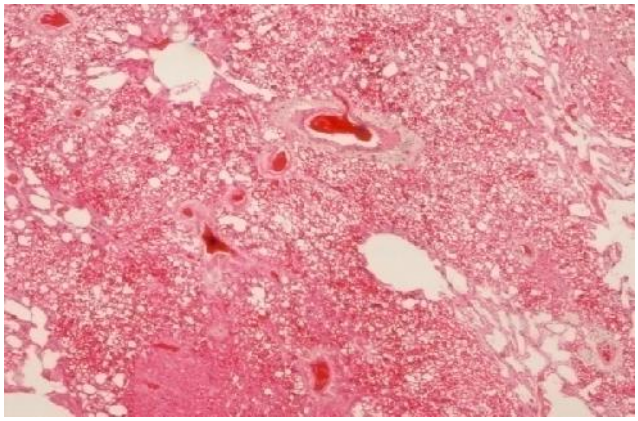

Plate 2: Histological lesions presentation of lungs infected by MG at 100X magnification

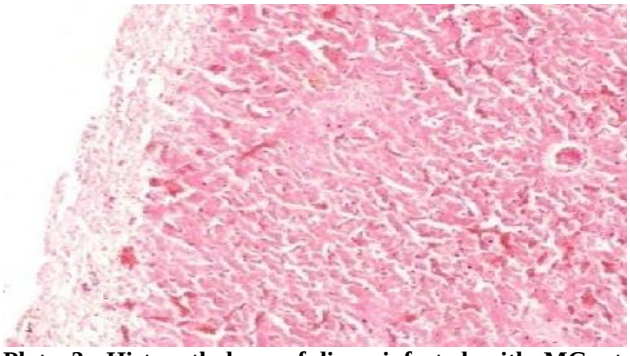

Plate 3: Histopathology of liver infected with MG at 100X magnification

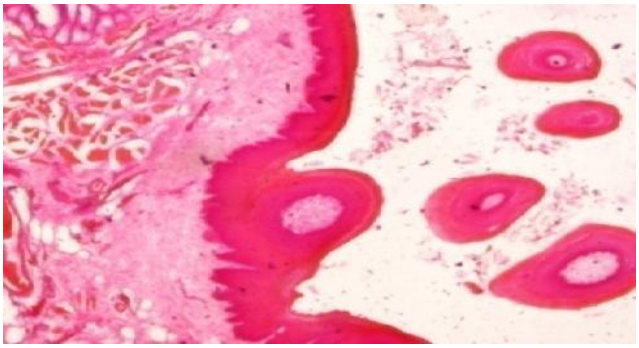

Plate 4: Histological lesions in ovary infected by MG at 100X magnification

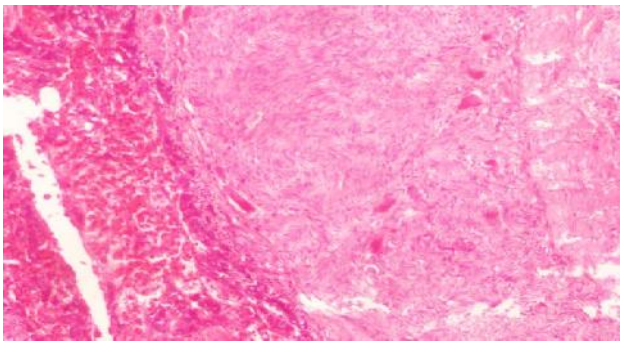

Plate 5: Histological lesions with epithelium disruption in oviduct of MG infected birds at 100X magnification

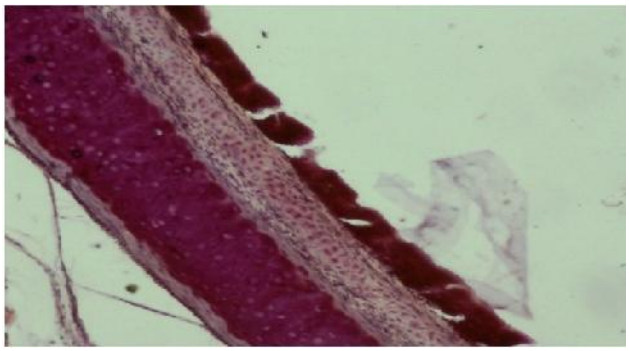

Plate 6: Immunohistochemical presentation of MG infected trachea at 100X magnification

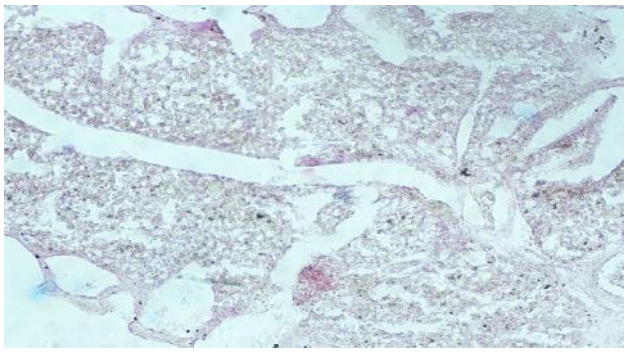

Plate 7: Immunohistochemical presentation of MG infected lung at 100X magnification

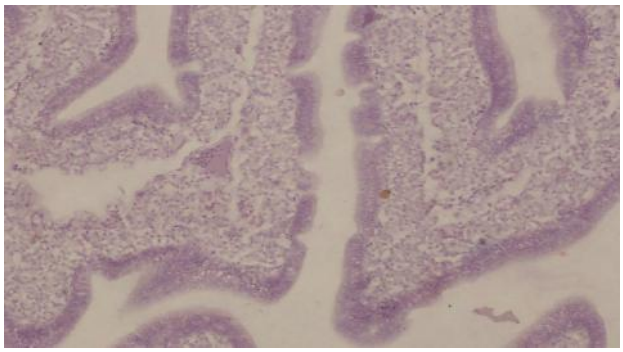

Plate 8: Immunohistochemical presentation of MG infected oviduct at 100X magnification

Plate 9: Immunohistochemical presentation of MG infected oviduct at 100X magnification

DISCUSSION

Mycoplama gallisepticum (MG) is chiefly encountered in respiratory tract and organs of reproductive tract (Buyuktanir *et al.*, 2008; Jenkins *et al.*, 2007). Three different routes of MG infection under experimental conditions were compared on the basis of clinical signs, post mortem findings, histological lesions and immunohistochemistry.

Clinical signs of CRD developed rapidly at early stage in intra-tracheal route followed by aerosol whereas mild to negligible in per-oral route. Signs included dullness, depression with slight anorexia, nasal discharge, coughing, lacrimation and open mouth breathing with

moist sounds. In corroboration findings were documented by Gharaibeh and Roussan (2008) and in contrast to present study mortality was recorded. Mortality observed was may be due to field conditions where chances of concurrent infections were more as compared to controlled experimental infection in the present study. Comparable observations recorded by Bajwa *et al*. (1992) that CRD was more severe in the presence of *E. coli.* Mild clinical signs in per-oral route observed in present controlled study were destruction of wall less MG by enzymes in gastro intestinal tract however, little portion escaped from enzymatic degradation caused focal infection.

In present study postmortem lesions were more pronounced in viscera of MG infected birds by intra-tracheal rout followed by aerosol and oral routes. Pattern of lesions was same but onset was early by intra-tracheal route. The pattern of development of lesions was in close association with research findings of Ley *et al*. (1997) and Gharaibeh and Roussan (2008). Air saculitis observed in MG infected birds was mild earlier, converted to thick cloudy form later on and was similar in fashion as described by Kleven *et al.* (2003) and Nascimento (2005). Lesions like congestion, consolidations and focal necrosis were present on the surface of lungs as reported by Yagihashi *et al.* (1988) and Rodriguez *et al.* (2000). In chronic condition characteristic extensive fibrosis on surface of lungs, heart, liver, thickening of air sacs and hypertrophy of internal lining of oviduct recorded was same as documented by Bajwa *et al.* (1992). Characteristic lesions including hemorrhages in the lumen of oviduct, marked thickening, enlarged adjacent lymph nodes and putrefied eggs were in corroboration to the results recorded by Nunoya *et al.* (1997).

Histological lesions were most consistent in lungs, trachea, liver and oviduct in acute pattern at early stage but became chronic in the late stage of MG infection. Sections of trachea revealed necrosis, hemorrhages, disruption of lining epithelium, marked leukocytes infiltration in sub-mucosal layer with thickening of mucous glands. These observations were similar to the findings of Gaunson *et al.* (2000). Lungs showed congestion, hemorrhages, focal necrosis, leukocyte infiltration with polymorphs nucleated cells, emphysema and exudates in alveoli were recorded in present plan of study were in agreement with findings of Branton *et al*. (1984). Thick glandular epithelium with extensive leukocyte infiltration noticed by Nunoya *et al.* (1997) was comparable with present planned experiments.

On immunohistochemical examination highest presence of antigen was confirmed in viscera by intra-tracheal route. Lungs showed positive immune reactions at early stage and in air vesicle spaces/cytoplasm of macrophages late during infection. These findings were similar to observations of Radi *et al*. (2000) determined by avidin-biotin-immunoperoxidase technique for detection of MG. In contrary to the results of Gaunson *et al.* (2000) no antigen was detected in trachea in any bird instead of having lesions. The reason for absence of reaction in present study may be due to MG attached to epithelium of trachea which sloughed. Positive immunohistochemical reaction in lumen of oviduct, salpingitis and hemorrhages observed were almost same as reported by Nunoya *et al*. (1997). The presence of antigen within the tissue confirmed that the antigen was the same as inoculated in excremental birds for documenting pathogenesis. The presence of antigen within the cells indicated that the MG was capable to enter into non phagocytes. In this way MG can evade itself from host immune response. It provides the evidence that chemotherapy fails in complete eradication of MG from the flock as it escapes from the action of antibiotic that having no access inside the cells. It ultimately leads to chronic carrier form for rest of life.

Intra-tracheal and aerosol routes are more effective for induction of disease during experimental studies. Disease produced adapted similar course, pattern of morbidity and severity as observed in field outbreaks with the difference of having no mortality. Immunohistochemistry is optimized for the first time in Pakistan for MG detection and found to be a reliable effective tool for the detection of MG antigen within the tissue affected by disease.

REFERENCES

Ahmad, A., M. Rabbani, T. Yaqoob, A. Ahmad, M. Z. Shabbir and F. Akhtar (2008). Status of IgG antibodies against *Mycoplasma gallisepticum* in nonvaccinated commercial poultry breeder flocks. The J. Anim. Plant Sci. 18(2-3): 61-63.

Anonymous. (2008). Avian mycoplasmosis (*Mycoplasma gallisepticum*, *Mycoplasma synoviae*), in OIE Terrestrial Manual. Pp: 482-496.

Bajwa, N. Z., M. Siddique, and M. T. Javed (1992). Pathogenesis of *Eschericha coli* in previously *Mycoplasma gallisepticum* infected layer chicks. J. Islamic Acad. Sci. 5: 123-126.

Bancroft, J. D. and M. Gamble (2002). Theory and practice of histological techniques. 5th. Ed. Edinburgh. Churchill Livingstone Pub. Pp: 593-620.

Bencina, D. and D. Dorrer (1984). Demonstration of *Mycoplasma gallisepticum* in tracheas of healthy carrier chickens by fluorescent-antibody procedure and the significance of certain serologic tests in estimating antibody response. Avian Dis. 28: 574- 578.

Bradbury, J. M. (1998). Recovery of Mycoplasmas from birds, In: Mycoplasma Protocols. (Miles, R. J.

and R. A. J. Nicholas; eds). Humana Press Inc, Totowa, N. J. 104: 45-51.

Branton, S. L., H. Gerlach and S. H. Kleven (1984). *Mycoplasma gallisepticum* isolation in layers. Poult. Sci. 63: 1917-1919.

Buyuktanir, O., T. Yildirim, C. Yakicier, O. Genc and N. Yurdusev (2008). A recombinant PvpA protein-based diagnostic prototype for rapid screening of chicken *Mycoplasma gallisepticum* infections. Vet. Microbiol. 129: 139-149.

Gaunson, J. E., C. J. Philip, K. G. Whithear and G. F. Browning (2000). Lymphocytic infiltration in the chicken trachea in response to *Mycoplasma gallisepticum* infection. Microbiol. 146(5):1223-1229.

Gharaibeh, S. and D. Al-Roussan (2008). The use of molecular techniques in isolation and characterization of *Mycoplasma gallisepticum* form commercial chicken in Jordan. Int. J. Poult. Sci. 7(7): 28-35.

Glisson, J. R. and S. H. Kleven (1984). *Mycoplasma gallisepticum* vaccination; Effects on egg transmission and production. Avian Dis. 28: 406-415.

Jenkins, C., S. J. Geary, M. Gladd and S. P. Djordjevic (2007). The *Mycoplasma gallisepticum* OsmC-like protein MG1142 resides on the cell surface and binds heparin. Microbiol. 153: 1455-1463.

Khalifa, K. A., M. B. Rahman and A. M. Mohammed (2008). Isolation, identification and molecular detection of *Mycoplasma gallisepticum* in poultry breeder poultry farm in Khartoum state, Sudan. Sudan J. Vet. Res. 23: 65-71.

Kleven, S. H. (2003). Mycoplasmas in the etiology of multifactorial respiratory disease. Poult. Sci. 77: 1146-1149.

Ley, D. H (2003). Mycoplasma gallisepticm infection. In: Disease of Poultry, Saif Y. M., Barnes H. J., Glisson J. R., Fadly A. M., McDougald L. R. & Swayne D. E., Eds. Iowa state University Ress, Ames, Iowa, USA, 722-744.

Ley, D. H. and H. W. Yoder (1997). *Mycoplasma gallisepticum* infection. In: Diseases of Poultry. 9th ed. Y. M. Saif 194-207.

Nascimento, E. R., V. L. A. Pereira, M. G. F. Nascimento and M. L. Barreto (2005). Avain mycoplasmosis update. Rev. Bras. Cien. Avic. 7(1): 1-9.

Nunoya, T., K. Kanai, T. Yagihashi, S. Hoshi, K. Shibuya and M. Tajima (1997). Natural case of salpingitis apparently caused by *Mycoplasma gallisepticum* in chickens. Avian Pathol. 26(2): 391-398.

Radi, Z. A., D. W. Trampel, B. S. Smith, R. F. Rosenbusch and F. Goll (2000). Immunohistochemical detection of *Mycoplasma gallisepticum* antigens in turkey respiratory tissues. Avian Dis. 44: 399-407.

Razin, S., D. Yogev and Y. Naot (1998). Molecular biology and pathogenicity of mycoplasmas. Microbiol. Mol. Biol. Rev. 62(4): 1094-1156.

Rodriguez, F., J. Sarradell, J. B. Poveda, H. J. Ball and A. Fernandez (2000). Immunohistochemical characterization of lung lesions induced experimentally by Mycoplasma agalactiae and Mycoplasma bovis in goats. J. Comp. Pathol. 123: 285-293.

Rottem, S. (2003). Interaction of mycoplasmas with host cells. Physiol. Rev. 83(2): 417-432.

Sankar, S., G. K. Nair, M. Mini and H. M. Harshan (2012). Polymerase chain reaction based detection of *Mycoplasma gallisepticum* in chicken. J. Ind. Vet. Assoc. 10(1): 5-8.

Yagihashi, T., T. Nunoya and M. Tajima (1988). Pathogenicity for chickens of six strains of *Mycoplasma gallisepticum* isolated from various birds. Avian Pathol. 17(3): 725-729.