Vet.Med.J., Giza. Vol.53, No.1. (2005):143-152.

THE APPLICATION OF A RECENT TECHNIQUE FOR DIAGNOSIS OF MYCOPLASMA GALLISEPTICUM INFECTION FROM MIGRATORY QUAIL

LAILA, M. EL-SHABINY, MONA, M. SHAKER, AND SAHAR, E. OUDA.

Mycoplasma Department, Animal Health Research Institute, Dokki, Giza.

Received: 1. 11. 2004

Accepted: 14. 12. 2004

SUMMARY

Twenty two Mycoplasma isolates were recovered from 120 Coturuix coturuix quails at Ismailia governorate. Identification and serotyping of these isolates revealed 9 *M. gallisepticum* (MG), 8 M. gallinarum, and 5 M. pullorum.

Experimental infection was designed where 10 chicks (one week old) were inoculated intranasally with 0.2 ml 10^7 CFU/ml *M. gallisepticum* isolated from quails, 10 chicks were inoculated with M. gallisepticum S6 strain and 10 chicks were considered as control negative.

Post mortem (PM) lesions were milder in chicks inoculated with M. gallisepticum isolated from quail compared to those inoculated with M. gallisepticum S6 strain and presented by congested lungs and liver, turbidity, and thickening of air sacs.

Histopathological examination showed that the lungs of chicks infected with *M. gallisepticum* isolated from quail showed oedema with mononuclear leucocytic inflammatory cells infiltration in the interlobular connective tissue stroma. The grade of histopathological examination of tracheas of chicks infected with M.G. isolated from quail showed local lymphoid cell aggregation of leucocytic inflammatory cells in the lamina propria underneath the epithelial mucosa was mild.

Mycoplasma gallisepticum could be reisolated and identified from challenged. Serologic antibody response was less in sera of chicks infected with *M. gallisepticum* isolated from quails.

Polymerase chain reaction confirmed that the Mycoplasma isolates were M. gallisepticum.

INTRODUCTION

M. gallisepticum (MG) is a major avian pathogen which causes chronic respiratory disease (CRD)

in chickens resulting in substantial economic losses to poultry producers throughout the world. CRD causes significant reduction in egg production, hatchability, and down grading of carcasses (Papazisi et al., 2002). However, Mycoplasma infection was reported in quails and ducks and should be considered not only as a potential source of transmission to other birds but also as a potential selection pressure for the generation of new variant and possibly pathogenic strains (Cookson and Shivaprasad, 1994).

Ley et al. (1997) reported an ongoing outbreak of conjunctivitis in free-ranging house finches caused by MG. They also isolated MG from blue jay and gold finches with conjunctivitis. Farmer et al. (2002) concluded that song birds were susceptible to MG infection but the prevalence was lower than in house finches.

Furthermore, Pillai et al. (2003) studied the epidemiology of MG isolated from house finch (Carpodacus Mexicans). Butcher (2004) stated that one of the potential means of transmission of MG is the spread by wild birds which demonstrates the need to wild-bird proof poultry houses.

The present study was conducted to assess infection of chicken with quail MG strain and its pathogenicity to chicken through applying an experimental infection and identification of the isolated Mycoplasmas by standard culture, polymerase chain reaction techniques, and titration of sero conversion.

MATERIALS AND METHODS

Mycoplasma isolates and antisera:

1- Twenty two Mycoplasmas isolated from lung, trachea and air sacs of 120 Quternix quails collected by hunting from different localities in Ismailia governorate. 9 M. gallisepticum, 8 *M. gallinarum*, and 5 *M. pullorum.*

2- Mycoplasma type cultures and reference antisera were kindly obtained from Dr, Shin Diagnostic Lab. Cornell University Ithaca N.Y. USA.

Culture Conditions

The isolates were cultured on pplo broth and agar (Razin, 1978) and propagated as described by Razin and Tully (1983). Purification was as mentioned by Tully (1983), genus determination was according to Freundt et al., (1973), maintenance after Leach (1983). Biochemical characterization was as described by Erno and Stipkovits (1973). Immunological identification was conducted using growth inhibition test after Clyde (1983).

Serological tests:

Serum plate agglutination test (SPA) was applied according to Kleven and Yoder (1989) using MG stained antigen of Intervet.

Experimental Infection of Chicks:

As shown in Table (1) thirty; one day-old chicks proved to be free from Mycoplasma by culture

and serological examination. At one week of age the chicks were divided into 3 groups of 10:

Group (1) was inoculated intranasally (I/N) with 0.2 ml MG quail isolate 10^7 (CFU/ml) broth culture which was isolated from quails.

Group (2) inoculated with 0.2 ml of MG S6 strain 10^7 (CFU/ml) broth culture as described by Kuba et al. (1968).

Group (3) was considered a control non infected group.

• Clinical and post mortem examination were carried out and recorded through the period of the experiment (one month).

• Reisolation of the inoculated MG was done every week.

Serological examination:

Blood samples from all infected birds were tested by SPA for antibodies against MG according to Kleven and Yoder (1989).

Histopathological examination:

At post mortem the trachea and lung were removed from each bird for histopathological examination as described by Bancroft et al. (1996).

Polymerase chain reaction (PCR):

Applied for one isolate of *M. pullorum* and 2 isolates of MG isolated from quails, 2 MG isolates reisolated from chicks and MG S6 strain according to Kempf et al. (1993).

DNA extraction:

This was carried out according to Kellog and Kwok (1990) and Blanchard et al. (1991). Oligonucleaotide amplification was conducted by PCR using forwarded primer (5\ TAACTATCGCATGAGAATAAC3\) and a reverse primer (5\GTTATTCAAATGGTACACAG-3\), PCR was performed on a Perkin Elmer cetus thermal cycler model 2400. Following an initial denaturation steps at 90°C for 1 min., the reactions were run for 40 cycles of: being 95°C for 15 seconds, 60°C for 20 sec. and 75°C for 15 seconds. An additional cycle 95°C for 15 seconds, 60°C for 45 sec. and 75°C for 5 minutes was included as a final step.

Analysis of PCR amplified products:

Alliquots of (10 µl) and also the molecular weight marker Qx Hae III digest from 72 bp to 1.353 Kbp. (Finexyme Finland), were analysed by gel electrophoresis on a 2% agarose gel. DNA was visualized by UV transilluminator and photographed after ethidium bromide staining.

RESULTS

Clinical signs:

• Chicks of group (1) infected with MG isolated from quail showed slight emaciation, ruffled feather, sneezing and watery nasal discharge.

- Chicks of group (2) infected with MG S6 strain showed emaciation, ruffled feather, depression, rales, sneezing, watery nasal discharge, and chough more severe than in group (1).

- Chicks of group (3) the control negative group revealed no apparent changes.

Post mortem lesions:

- As recorded in Table (2) chicks infected with MG isolated from quail (group 1) showed slight congestion of lungs, liver, slight thickening and turbidity of air sacs.

- Chicks infected with MG S6 strain (group 1) showed severe congestion of lungs and liver, severe thickening and casiation of the air sacs.

- The chicks of the control non infected group (3) had no PM lesions.

Reisolation and Sero Conversion: Results recorded in Table (3) showed that MG could be reisolated from 6 out of 10 chicks inoculated with MG isolated from quails and 8 out of 10 chicks infected with MG S6 strain, while no Mycoplasmas could be isolated from chicks of the control group. It was also noticed that reisolation of MG began from the first week of infection in all infected groups

Serologic antibody response was higher in chicks inoculated with MG S6 strain compared with those inoculated with MG isolated from quail.

Histopathological examination:

The results recorded in Plate 1(A) showed that the lung of chicks infected with MG strain isolated from quails had oedema with mononuclear leucocytic inflammatory cell infiltration in the interlobular connective tissue stroma (grade I) while lungs infected. with MG S6 strain (Plate 1B) showed focal lymphoid cell aggregation in the lung lobules (grade II).

Trachea of chicks infected with MG isolated from quail (Plate 1D) showed focal aggregation of the leucocytic inflammatory cells in the lamina propria underneath the epithelial mucosa (grade I), while the trachea of chicks infected with MG S6 strain (Plate 1E) showed desquamated lining mucosal epithelium with massive number of leucocytic inflammatory cells in the underlying lamina propria (grade II). Regarding the lung and trachea of chicks of the control negative group, they showed the histological structure of the different layers (grade 0), as shown in Plate 1(C & F).

The results of Polymerase chain reaction:

The electrophoresis of PCR products revealed that, all MG strains (Lanes, 3-7) showed a specific band at 330bp. Meanwhile M. pullorum (Lane, 2) didn't show any reaction as shown in Figure (1).

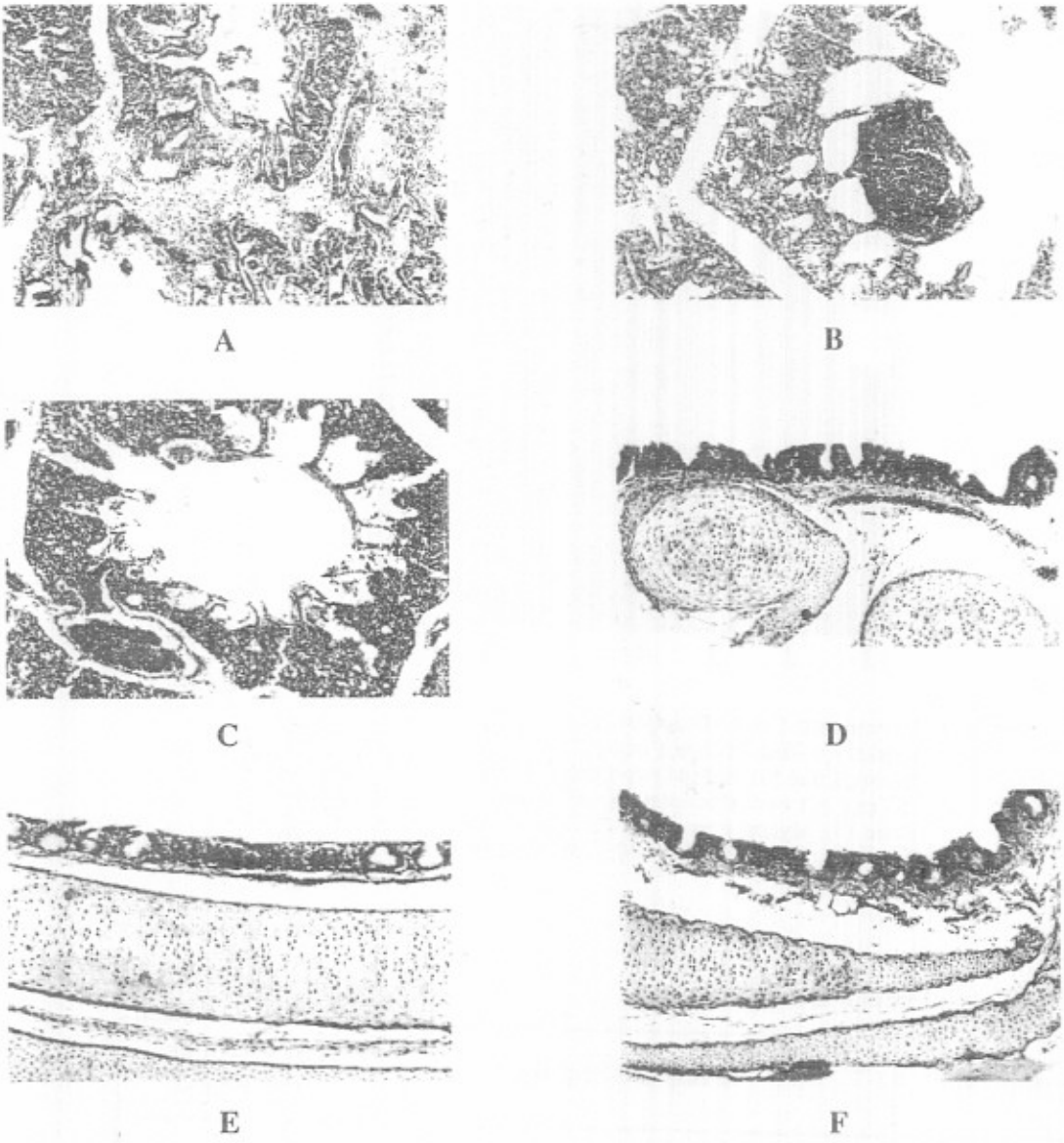

Plate I: (A) Lung Grade I: showing oedema with mononuclear leucocytic inflammatory cells infiltration in the interlobular connective tissue stroma. (B) Lung Grade II: showing focal lymphoid cells aggregation in the lung lobules H&E x40. (C) Lung Grade 0: showing the histological structure of the lung lobules and interlobular stroma. (D) Trachea Grade I: showing focal aggregation of leucocytic inflammatory cells in the lamina propria underneath the epithelial mucosa. (E) Trachea Grade II: showing desquamated lining mucosal epithelium with massive number of leucocytic inflammatory cells in the underlying lamina propria H&E x40. (F) Trachea Grade 0: Trachea of chicken ìControl Negativeî showing the histological structures of the different layers.

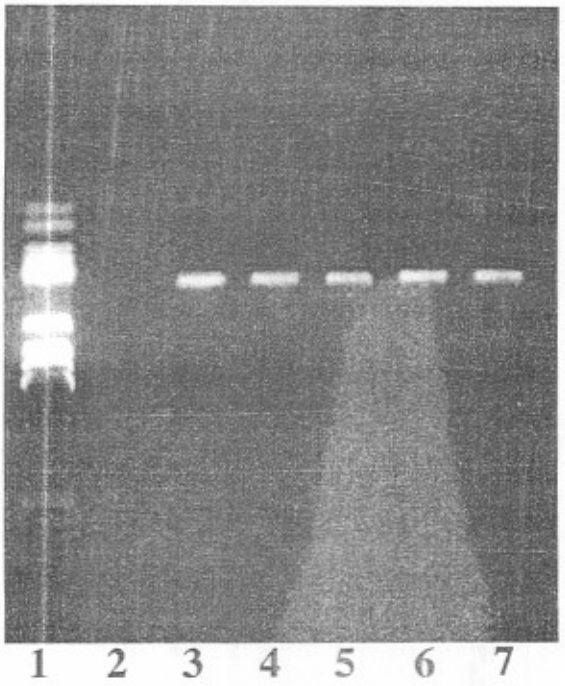

Figure 1: Polymerase Chain Reaction results: Lane (1) Molecular weight marker Qx Hae III digest from 72 bp.-1.35 Kbp.; Lane (2) M. pullorum; Lane (3) and (4) two Quail M. gallisepticum strains; Lane (5) and (6) two Mycoplasmas reisolated from infected chickens; Lane (7) Mycoplasma isolated from chickens infected with MG S6 strain. Lanes 3,4,5,6, and 7 showing specific bands at 330 bp.

Table (1): Experimental Design.

Group No.	Group Description	No. of Chicks Examined
Group (1)	Chicks inoculated with quail MG strain	10
Group (2)	Chicks inoculated with MG 56 strain	10
Group (3)	Negative Control group	10
Total	--	30

Table (2): Post Mortem lesions Results

Organ / Group No.	Lung	Liver	No. of Chicks Examined
Group (1)	Slight congestion	Slight congestion	Slight thickening & turbidity
Group (2)	Severe congestion	Severe congestion	Severe thickening & castiation
Group (3)	Normal	Normal	Normal

Table (3): Reisolation of MG and Sero Conversion Results

No. of Chicks / Group No.	Examined	Reisolated	Percentage	Sero Conversion SPA*
Group (1)	10	6	60%	++
Group (2)	10	8	80%	+++
Group (3)	10	0	0%	--

• ++ Low antibody response
• +++ High antibody response
• * SPA Serum Plate Agglutination

DISCUSSION

The adaptation of MG to a free-flying avian species presents potential problem for the control of Mycoplasmosis in commercial poultry (Luttrell et al., 2001).

Therefore, the objectives of the present study were to determine if chickens could be infected with quailís MG strain and its pathogenic effect on chickens.

The present study reported mild gross lesions, mild clinical symptoms and histological changes of lung of chicks inoculated with quails MG strain presented by oedema with mononuclear leucocytic inflammatory cell infiltration in the interlobular connective tissue stroma and in the trachea focal aggregation of the leucocytic inflammatory cells in the lamina propria underneath the mucosa.

In addition, low rate of reisolation determined by

culture and lower seroconverison in chicken inoculated with MG quail strain. These findings indicated that it could infect chicken in a lower degree than in chicken infected with MG S6 strain. These results agree with those of O'connor et al. (1999) who stated that MG isolated from wild house finches may infect domestic poultry species but causes only mild disease and was less virulent than MG R-strain. Our results also coincide with those of Stalknecht et al. (1998) who mentioned that chickens could be infected by contact with naturally infected house finches as determined by culture and PCR methods. Clinical and histopathological signs were mild.

The data of the present study clarified that PCR for Mycoplasmas isolated from quails and reisolated from chickens infected with them gave a band at 330 bp indicating that they are MG strains. This finding agrees with that of Pillai et al. (2003) who also proved by PCR that the Mycoplasmas he isolated from finch and those reisolated from chickens were MG strains. However, he reported relatedness and variability of MG finch isolates to those of chicken and turkey.

On the other hand, Mc Martin et al. (1996) carried out an experimental infection of chukar partridge with MG and the disease was confined to conjunctiva and upper respiratory tract. Also, Shaker (2001) applied an experimental infection to quails and Mollards ducks with Mycoplasma isolated from these birds which revealed PM lesions including congestion of lung and liver, turbidity and thickening of air sacs only in case of infection with MG and *M. anatis*.

Also, Kollias et al. (2004) applied an experimental infection of house finches with MG finch strain and recorded the pathogenic effect.

The results of the present study recorded that quails MG strains were initially identified by a strong growth inhibiting response identical to that produced by MG S6 strain which was confirmed by PCR. This finding denoted to the importance of PCR as a rapid sensitive and specific technique for detection of Mycoplasma.

It is recommended that further study should be applied using PCR restriction fragment length polymorphism to clarify relatedness and variability of these isolates to those isolated from chickens.

It is also recommended to apply the appropriate biosecurity measures to prevent migratory birds as an important mean of transmission of Mycoplasma infection to chickens and turkeys.

REFERENCES

Bancroft, J.D.; Stevens, A. and Turner, D.R. (1996): Theory and practice of histological techniques 4th ed. Churchill

Livingstone, New York.

Blanchard, A.; Gautter, M. and Mayaoe, V. (1991): Detection and Identification of Mycoplasmas by amplification of rDNA. Fems Microbiology letters 81:37-42.

Butcher, F.D (2004): Mycoplasma gallisepticum ñA continuing Problem in Commercial Poultry. Vet. Med. Large animal clinical sciences Dept. Series University of Florida, VM 130.

Clyde, W.A. (1983): Methods in Mycoplasma Morphology: Growth inhibition test. In Methods in Mycoplasmology, Vol.1, 405, Academic Press, New York.

Cookson, K.C. and Shivaprasad. H. (1994): Mycoplasma gallisepticum infection in Chukar Partridges, Pheasants and Pea fowl. Avian Dis. 38:914-921.

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

Farmer, K.L.; Hill, G.E. and Roberts, S.R. (2002): Susceptibility of a native Population of House Finches To Mycoplasma gallisepticum. J. Wild Dis. 38(2):282-286.

Freundt, E.A.; Anderson, B.E.; Erno, N.; Kunze, M. and Black, F.T. (1973): The sensitivity of Mycoplasmatales to Sodium Polyanethol sulphate and Digitonin. Z. bl. Back. Parasit: Infehkter. Hyg. L. Abt. Drig. A. 275:104-112

Kellog, D.E. and Kwok, S. (1990): Detection of human immunodeficiency virus in PCR protocols: a guide to methods and application. 339-343.

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

Kleven, S.H. and Yoder, H.W. (1989): Mycoplasmosis In: A laboratory manual for the isolation and identification of avian pathogens,3rd ed. H.G. Purchase, L.H. Arp, C.H. Domermuth, and J.E .Pearson, eds. Kendall ,Hunt, Dubuque, Iowa. Pp:57-62.

Kollias, G.V.; Sydenstricker, K.V.; Kollias, H.W.; Ley, D.H.; Hosseini, P.R.; Connolly, V. and Dhondt, A.A. (2004): Experimental infection of house finches with Mycoplasma gallisepticum. J. Wild Dis. 40(1):79-86.

Kuba, N.; Hashimota, K.; Sato, T.; Sochi, I.; Inoguchi, T. and Komatsu, M. (1968): Studies on experimental Mycoplasmosis in chickens. Clinical observation of chickens inoculated organisms. Japanese J.Vet.Sci.12:315-321.

Leach, R.H. (1983): Preservation of Mycoplasma culture and culture collection in Mycoplasmology. Vol.11 Edited by Shamuell Razin and Joseph G. Tully 1983. Acad. press. New York.

Ley, D.H., Berkhoff, J.E. and Levisohn, S. (1997): Molecular epidemiologic investigations of Mycoplasma gallisepticum conjunctivitis in song birds by random amplified polymorphic DNA analysis. Emerg. infect.Dis.3 (3):375-380.

Luttrell, M.P.; Stalknecht D. E.; Kleven, S.H.; Kavanaugh, D.M.; Corn, J.L. and Fischer, J.R. (2001): Mycoplasma gallisepticum in house finches (carpodacus mexicans) and other wild birds associated with poultry production facilities. Avian Dis. 45(2):321-329.

McMartin, A.J.; Damassa, W.D.; McKeen, M.; Reed, D.; Barbara, D. and Lam, K.M. (1996): Experimental reproduction of MG disease in chukar partridges (Alectoris graeca). Avian Dis. :408-416.

O'Connor, R.J.; Turner, K.S.; Sander, J.K.; Kleven, S.H.; Brown, T.P.; Gomez, L. and Dine, J.T. (1999): Pathogenic effects on domestic poultry of a MG strain isolated from a wild house finch. Avian Dis. (4):640-648.

Pa,)azisi, I.; Silbart, L.K.; Frasca, S.; Rood, D.; Liao, X.; Gladd, M.; Javed, M.A. and Geary, S.J. (2002): A modified Live Mycoplasma gallisepticum vaccine to protect chickens from respiratory disease. Vaccine 20:3709-3719.

Pillai, S.R.; Mays, H.L.; Ley, D.H.; Luttrell, P.; Panangala, V.S.; Frmer, K.L. and Roberts, S.K. (2003): Molecular variability of house finch Mycoplasma gallisepticum isolates as revealed by sequencing and restriction fragment length polymorphism analysis of the PVPA gene. Avian Dis. 47(3): 640-648.

Razin, S. (1978): Methods in Mycoplasmology. Mycoplasma characterization. Academic Press, New York.

Razin, S. and Tully, J.G. (1983): Methods in Mycoplamology. Acad. Press, New York (11):197-204.

Shaker, M.M. (2001): Occurrence of Mycoplsma in some migratory birds. Benisuef Vet. Med. J.(2):165-172.

Stalknecht, D.E.; Luttrell, M.P.; Fischer, J.R. and Kleven, S.H. (1998): Potential for transmission of finch strain of Mycoplasma gallisepticum between house Finches and chicken. Avian Dis. 42(2):352-358.

Tully, J.G. (1983): Cloning and filtration techniques for Mycoplasmas. Methods in Mycoplasmology, Vol. I, Acad. Press, New Yorl: 173-177.