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# Survey of Layer Flocks Contamination to *Mycoplasma gallisepticum* in East Azerbaijan Province by Rapid Slide Agglutination (R.S.A) Method

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**Abstract:** The objective of this study was to evaluation of R.S.A. method efficiency in distinguishing *M. gallisepticum* and contamination rate of MG in Iran. In this study totally 300 serum samples from 20 farms collected and sent to laboratory. In lab, samples centrifuged at 2500 rpm for 5 min. Separated serums kept in Bain Marie at 56ºC. Then 50 µ of serum are mixed with 50 µ RSA-MG antigen on the slide and were assayed under light. Of 300 serum samples, 52 samples (17.3%) were positive, 10 samples (3.3%) were suspicious and 238 samples (79.3%) were negative reported. Finally, primarily can be conclude that R.S.A method is specific method for detection of *M. gallisepticum* and has minimum Err with maximum sensitivity. Secondarily can be conclude that contamination rate of *M. gallisepticum* in Iran is higher than standard levels and must be take measures in this field.

**Key words:** Layer flocks, *Mycoplasma gallisepticum*, R.S.A, Tabriz

## INTRODUCTION

*Mycoplasma gallisepticum* is the most economically significant mycoplasmal pathogen of poultry. *M. gallisepticum* infections can cause significant economic losses on poultry farms from chronic respiratory disease reduced feed efficiency, decreased growth and decreased egg production. The carcasses of birds sent to slaughter may also be downgraded. *M. gallisepticum* infections are notifiable to the World Organization for Animal Health (OIE, 2011). This organism has been eradicated from most commercial chicken and turkey breeding flocks in the United States; however, it remains endemic in many other poultry operations. Since 1994, *M. gallisepticum* conjunctivitis has become an emerging disease in finches. This disease has been responsible for major declines in house finch populations in the eastern U.S., and was recently reported in western house finch populations. *M. gallisepticum* can also affect other finch species, although its impact has not been as severe.

**Etiology:** Avian mycoplasmosis can be caused by several species of Mycoplasma (class Mollicutes, order Mycoplasmatales, family Mycoplasmataceae) including *Mycoplasma gallisepticum*, *M. synoviae*, *M. meleagridis* and *M. iowae*. *M. gallisepticum* is the most important pathogen in poultry. It also causes disease in other avian species. *M. gallisepticum* infections are also known as Chronic Respiratory Disease (CRD) of chickens, infectious sinusitis of turkeys and house finch conjunctivitis.

Several strains of *M. gallisepticum* have been reported, including the R (poultry), P (psittacine) and house finch strains. Strains may vary greatly in their pathogenicity for different species of birds. In one study, budgerigars developed severe disease after experimental infection with the R strain of *M. gallisepticum* but not the house finch strain.

**Species Affected:** *Mycoplasma gallisepticum* causes disease in chickens, turkeys, and game birds including pheasants, chukar partridges, bobwhite quail, Japanese quail and peafowl. The organism has also been isolated from ducks and geese, as well as yellow-naped Amazon parrots, pigeons and greater flamingos. It has been found in wild peregrine falcons in Spain (Farmer *et al*., 2005; Kollias *et al*., 2004).

Since 1994, *M. gallisepticum* epidemics have been reported in house finches (*Carpodacus mexicanus*) in the U.S. This organism has also been confirmed by culture or Polymerase Chain Reaction (PCR) in American goldfinches (*Carduelis tristis*), purple finches (*Carpodacus purpureus*), eastern tufted titmice (*Baeolophus bicolor*), pine grosbeaks (*Pinicola enucleator*), evening grosbeaks (*Coccothraustes vespertinus*) and a captive blue jay (*Cyanocitta cristata*). PCR-positive mourning doves (order Columbiformes) have also been reported, but these birds remained seronegative and culture negative, and may have been infected by a related species of Mycoplasma. Other passerine *species* have tested positive by serology. House sparrows (*Passer domesticus*) and budgerigars (*Melopsittacus undualtus*) have been infected experimentally with some strains.

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**Geographic distribution:** *M. gallisepticum* can be found worldwide. In the United States, this organism has been eradicated from most commercial chicken and turkey breeding flocks, but remains present in other poultry operations. Beginning in 1994, *M. gallisepticum* epidemics associated with conjunctivitis were reported in house finches throughout the eastern U.S. Infected birds have recently been reported in house finch populations in the western U.S (Bozeman *et al*., 1984).

**Transmission:** *M. gallisepticum* is transmitted during close contact between birds as well as on fomites. Aerosol spread occurs over short distances and can be responsible for transmission within a flock. *M. gallisepticum* is also transmitted vertically in eggs.

Chronic respiratory disease is caused by *Mycoplasma gallisepticum* (MG) in chickens, resulting in reduced feed conversion egg production and significant downgrading of carcasses at slaughter. Transmission can occur through eggs or by inhalation of contaminated airborne droplets, resulting in rapid disease transmission throughout the flock. MG is a highly infectious respiratory pathogen affecting poultry. The clinical signs associated with MG infection in chickens include respiratory rales, nasal discharge, coughing, and occasionally conjunctivitis (Saif *et al*., 2003). Programs for control and eradication of the pathogen from breeder flocks are traditionally based on serological testing and isolation of the organism. However, it is difficult to diagnose MG infections in poultry flocks on the basis of clinical signs, routine culture procedures and commonly used serology (Mallinson, 1983; Yoder, 1986). The diagnosis of MG infection traditionally has been done by serology. Some of the disadvantages of serological methods are false-positive and false-negative reactions due to interspecies cross-reactions and nonspecific reactions. Moreover, there are some *Mycoplasma* species, e.g. *Mycoplasma iowae* showing antigenic heterogeneity and poor immune response that makes the development of reliable serological methods for detection more difficult (Jefferey *et al*., 1995). *In vitro* isolation of the organisms is usually used to confirm serological results. However, confirmation of an isolate by growth inhibition requires considerably additional time and monospecific antisera which are expensive. These techniques are time consuming, labor intensive and there are chances for false negative and false positive results. (Fan *et al*., 1995). Recently, the rapidly developing nucleic acid-based molecular biological techniques have been employed and PCR based methods have proved to be excellent tools for Rapid and effective identification of *Mycoplasma strains*. (Wang et al., 1997;

## MATERIALS AND METHODS

This study conducted in 20 important farms of east Azerbaijan province, Iran during April-July 2011. From each farm amount 15 blood samples ($2^{CC}$) achieved by chance and then sent to laboratory immediately within 24 hours. About all farms, vaccination program was finished and samples 6 month after latest vaccination were obtained because, of false positive results can be note to the killed oil vaccines. In lab, samples centrifuged at 2500 rpm for 5 minute. Separated serums kept in Bain Marie at $56^{\circ C}$. Then 50µ of serum are mixed with 50µ RSA-MG antigen on the slide and were assayed under light. If serum sample not formed agglutination reaction within 2 minutes, serum is reported negative otherwise is reported Suspicious or intermediate. In this condition, 1/8 dilution of serum is tested with antigen again. If in second time

Table 1: Data obtained from RSA test from 20 farm

| No. of flock | Age of flock (week) | No. of samples | positive | Suspicious | Negative | RSA test results at the 1/8 dilution |
|---|---|---|---|---|---|---|
| 1 | 30 | 15 | 0 | 0 | 15 | 0 |
| 2 | 52 | 15 | 3 | 2 | 10 | 3 |
| 3 | 58 | 15 | 7 | 0 | 8 | 7 |
| 4 | 70 | 15 | 5 | 0 | 10 | 5 |
| 5 | 45 | 15 | 0 | 5 | 10 | 0 |
| 6 | 40 | 15 | 0 | 0 | 15 | 0 |
| 7 | 38 | 15 | 3 | 0 | 12 | 3 |
| 8 | 45 | 15 | 10 | 0 | 5 | 10 |
| 9 | 51 | 15 | 0 | 0 | 15 | 0 |
| 10 | 62 | 15 | 0 | 0 | 15 | 0 |
| 11 | 32 | 15 | 0 | 0 | 15 | 0 |
| 12 | 39 | 15 | 0 | 0 | 15 | 0 |
| 13 | 35 | 15 | 9 | 0 | 6 | 9 |
| 14 | 58 | 15 | 0 | 0 | 15 | 0 |
| 15 | 45 | 15 | 15 | 0 | 0 | 15 |
| 16 | 51 | 15 | 0 | 0 | 15 | 0 |
| 17 | 55 | 15 | 0 | 0 | 15 | 0 |
| 18 | 48 | 15 | 0 | 3 | 12 | 0 |
| 19 | 53 | 15 | 0 | 0 | 15 | 0 |
| 20 | 39 | 15 | 0 | 0 | 15 | 0 |
| Sum | | 300 | 52 | 10 | 238 | 52 |

Table 2: data obtained from RSA test from 20 farm in terms of percent

| | Frequency | % |
|---|---|---|
| Positive | 52 | 17.3 |
| Suspicious | 10 | 3.3 |
| Negative | 238 | 79.3 |
| Total | 300 | 100 |

agglutination be seen within 2 min, serum is reported positive (Allan and Gough, 1974). *Feberwee et al., 2005; Ben et al., 2005). The objective of this study was to evaluation of R.S.A. method efficiency in distinguishing M. gallisepticum and contamination rate of MG in Iran.*

## RESULTS AND DISCUSSION

*Data are showed in Table 1 and 2*. Based on tables, of 300 serum samples, 52 samples (17.3%) were positive, 10 samples (3.3%) were suspicious and 238 samples (79.3%) were negative reported. In the present study, RSA tests with commercial antigens demonstrates more positive results (17.3%) as compared to the isolation and identification by Atif and Najeeb (2007) which is about 9%. The most widely used serological test for MG monitoring is the rapid slide agglutination test. According to Roberts (1969), chickens infected with three different strains of MG always reacted serologically with the homotypic and the heterotypic RSA antigens. Thus the numbers of positive or suspicious chickens according to the two tests were significantly different. A similar result was reported by Lin and Kleven (1982) with strains K503 and K730 which were shown to differ serologically from classic MS strains such as A5969. In the RSA test, birds singly infected with the variant strain had high antibody titres against the homologous antigen and a variable but lower response against the other antigens. It is well established that antigen differences between the hemagglutinin of the field strain and the diagnostic strains may lead to false negative results. For mycoplasmas, the development of diagnostic tools is difficult due to the problem of antigenic proteins undergoing high frequency variation in typical strains as Levisohn *et al.* (1995) established that surface antigens of MG are subjected *in vivo* to rapid alteration in their expression. This variability may function as a crucial adaptative mechanism, enabling the organism to escape from the host immune defense and to adapt to the changing host environment at different stages of a natural infection. Thus, diagnostic tools should be able to cope with a wide spectrum of antigen presentations. Finally can be conclude that contamination rate in Iran is higher than standard levels and must be take measures in this field.

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