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ORIGINAL ARTICLE

Comparative Evaluation of Serological test in Diagnosis of *Mycoplasma gallisepticum* Infection in Iranian North-west rural Poultry

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ABSTRACT

Mycoplasma gallisepticum (MG) is one of the most important diseases in Iranian poultry industry and all over the world. Mortality, poor weight gain and increasing of feed conversion ratio (FCR) were seen in MG infected flocks, and causes economical losses particularly in broiler chickens that were used for meat production.

In this study, 3 broiler breeder rural farms located around Tabriz city of Iran were investigated during 2011-2012. In each farm 30 serum samples were obtained. The prevalence of Mycoplasma gallisepticum was studied by RSA and ELISA test. Our results indicated that the from 90 samples were tested, 38 (42.22%) of samples were positive in RSA, while 30 (33.33%) of samples were positive in ELISA.

It can be concluded that RSA and ELISA serological tests should be only used as screening in monitoring programs to detect MG in poultry flocks and positive results should be confirmed by routine microbiological tests. The higher rate of MG in broiler flocks indicated that these farms do not consider biosecurity and hygienic conditions.

Key words: *Mycoplasma Gallisepticum, RSA, ELISA, Serological methods, Broiler breeder.*

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INTRODUCTION

Mycoplasma gallisepticum (MG) is one of the most important disease in poultry production and also it is the causative agent of chronic respiratory disease in chickens [15]. MG infection causes significant economic losses in the poultry industry due to downgrading of carcasses at slaughter because of airsacculitis, treatment costs, and due to its effect on flocks performance [18], and reduction of egg production in chickens, turkeys and other avian species were reported [17]. MG infection mainly is transmitted through ovaries, and the MG-infected breeder flocks should be depopulated; hence, the preferred method for MG control is to maintain MG-free flocks [26]. However, in some situations such as multi-age production farms, maintaining the flocks free of MG may be difficult or impossible. Also MG infection is of high economic significance because of high morbidity and high mortality.

MG can be diagnosed by its different properties such as microbial culture, biochemical and serological properties [14, 17]. Serology is the only reliable tools for detecting the subclinical infection in the flock [2]. There are two major Serological methods, which were used for screening breeder farms in Iran, Rapid Serum Plate Agglutination (RSA), and Enzyme Linked Immunosorbent Assay (ELISA); however, there were differences in sensitivity and specificity of these methods.

Eradication is the most important control measure for MG infections in poultry production. Especially eradication of vertically transmitted agents, early detection of new infections is extremely important. For a long period, control and prevention programs were based on use of the rapid serum plate agglutination (RSA) test, Hemagglutination inhibition (HI) test, and culture. Recently, enzyme-linked immunosorbent assays (ELISAs) have been introduced.

There were some difficulties in use of serologic tests for Mycoplasma that has been described previously. Problems with the use of the RSA test, particularly when undiluted sera are tested, are: a) nonspecific reactions due to bad quality or freezing of the sera, b) properties of the antigen preparation, c) recent use of inactivated vaccines, and d) cross-reactions based on the antigenic relationship between MG, *Mycoplasma Synoviae*, and *Mycoplasma imitans* [6, 11, 22, 23, 27]. HI test is less sensitive but more specific than the RSA test. As the HI test is strain specific, it may not detect antigenic variants that differ from the strain used as antigen in the test (1). The ELISAs have been developed from a need to facilitate and automate Mycoplasma testing [1]. However, lack of specificity and/or sensitivity of ELISAs in the acute phase of infection has been reported [2,3,30,47].

RSA is used as the screening test because it's rapid, has high sensitivity, and low specificity, as well as being inexpensive. ELISA has been proved to have good sensitivity and more specificity compared to RSA [16]. Due to economic importance diagnosis and prophylaxis of avian mycoplasmosis have received attention, recently. According to Iranian Veterinary Organization rules control of MG is dependent on serologic screening results.

The aim of the present study was to compare performance of two serological methods (RSA and ELISA) in diagnosis of antibody responses of rural breeder farms against *Mycoplasma gallisepticum*.

MATERIAL AND METHODS

In this study, 3 broiler breeder rural farms located around North-west of Iran were Selected and investigated during 2011-2012. From each flock 30 chickens (a total of 90 samples) were randomly selected for blood collection, 4 ml of blood was collected aseptically from wing vein of each bird and then sera were separated and stored at -21°C until use for RSA and ELISA tests.

The RSA test was conducted with crystal violet stained *M. gallisepticum* commercial antigen obtained from Intervet Company. One drop antigen and one drop fresh serum was placed side by side with pipette in a glass plate and mixed well by stirring with glass rod, followed by rocking. Results were read within 2 minutes. In positive cases granules were formed slowly which could be seen during rocking. In the negative case, no such granules were formed. All RSA results were recorded.

The antibody against MG detected in serum from each flocks with commercial ELISA kit (IDEXX, USA). The procedure was followed according to the manufacturer instruction.

RESULTS AND DISCUSSION

Mycoplasma infections are important poultry disease that causes economical losses in poultry production. Purpose of this study was to investigate performance of two different serological tests in detection of antibodies against *Mycoplasma gallisepticum*. Sera samples were collected during 2011-2012 years.

The results of the serologic tests demonstrated that a certain level of false positive results can be expected in any test. Although the level of false-positive results varied between several serologic tests, for this reason it is not advisable only to rely completely on one test [7]. All MG diagnostic tests (especially serology) showed a lower sensitivity in the detection of infection with some MG and MS strains [7].

Serological Tests (RSA, and ELISA), results were presented in table 1. The results indicated the frequency of antibodies against MG detected by RSA, and ELISA. Results showed that 42.22% of total samples were taken (38/90) were positive in RSA. While in ELISA test 33.33% (30/90) of samples were positive.

Table-1: Comparison of RSA, and ELISA test for *Mycoplasma gallisepticum* diagnosis in rural poultry breeders

Farm Number	RSA (positive %)	Positive/total	ELISA (positive %)	Positive/total
1	40	12/30	30	9/30
2	33.3	10/30	26.66	8/30
3	53.33	16/30	43.33	13/30
Total	42.22	38/90	33.33	30/90

Comparison of the RSA and ELISA results in each of farms is presented in Table1, which shows number and percentage of positive samples. Number of positive samples in ELISA test was lower than RSA test in all flocks and in total.

Some studies reporting the preference of diluted sera compared with undiluted sera for the MG RSA tests [23]. Although the specificity of the MG RSA test increased when using the diluted serum, but the sensitivity of test was decreased.

It seems very unlikely that the relatively high number of false positives would be representative for the field situation. Several factors play a role in the high number of false-positive results in several tests: serum of birds that were recently infected with a heterologous Mycoplasma species, lack of heat inactivation, age of the birds and use of inactivated vaccines. Heat inactivation leads to the denaturation of nonspecific immunoglobulins and, in this way, can contribute to less nonspecific reactions, especially in the RSA test [23]. The OIE recommends the use of serological tests for avian mycoplasmosis only as screening tools in the diagnosis of flocks, not of individual birds. This recommendation is based on the presupposition that tests have different sensitivities and specificities (7, 20). Also researchers indicated that the screening programs that are only based on seroconversion may be inadequate for mycoplasmosis diagnosis and control [5], and some others suggested that the positive results obtained in RSA test should be confirmed by additional tests, such as HI, because of the lack of specificity and false positive results observed in RSA [12].

RSA show false positive results in screening flocks following use of inactivated or oily vaccines, contaminated sera, and cross reactions [19]. However, atypical infections with low immunogenic potential may cause false negative results. Some studies suggested the isolation methods should be used only in case of positive serological results [9]. In addition, the type of antibody detected by serological tests varies, while RSA detects IgM antibody found 3 to 5 days after infection, and which persists for 70–80 days, but the HI and ELISA tests detect IgG antibody found 7 to 10 after infection, and which persists for up to 6 months [4].

Our results indicated that *Mycoplasma gallisepticum* infection in broiler is prevalent around Although the results of our study in agreement with previous studies, but due to controlling rules that approved by Iranian Veterinary Organization, MG positive parent flocks were slaughtered and the higher rate of MG in rural broiler breeder flocks indicated that these farms do not consider biosecurity and hygienic conditions. Additionally, it was proved that the occurrence of *Mycoplasma gallisepticum* have a relationship with the sampling year, season and ages of chickens, which should be studied more in detail.

High prevalence rate of MG infection was reported previously by several studies in poultry farms [13, 21, 24]. Some researchers mentioned that the seroprevalence of MG infection was higher (33.3%) in female than in male (10.14%), which it is indicating that the female birds significantly ($p<0.05$) were more susceptible than male birds. Isolation and identification of MG in Ghaemshahr town in north of Iran, showed that 20% of broiler farms positive in case of Mycoplasma genus and 12 percent of farms positive in molecular tests. Also several researches was indicated that, regardless of the screening of broiler breeder farms and control of MG, still high prevalence of MG present in poultry farms of Iran (8, 10). Previous studies on broiler breeder farms in Iran indicated high sero-prevalence (21.4%) of MG [3, 25]. Also it was reported that the prevalence of MG infection was higher (56.21%) in female than in male (43.79%) [8, 25]. Our results indicated that positive samples were lower in ELISA (33.33%) than RSA (42.22%), and these results in agreement with previous studies [19]. Also, some researchers results offering that positive results obtained of RSA test should be confirmed by additional tests, such as HI, because of the lack of specificity observed in RSA. These findings indicate that use a confirmatory test like isolation of the agent by microbiological tests or using molecular assays are essential.

However, intensive nature of poultry farming provided opportunity for recycling of the pathogens due to population density. The other factors that contribute MG infection are poor ventilation, contamination of litters and no restriction on the movement of the technical personnel, visitors and such other persons as well as other biosecurity measures [21).

CONCLUSIONS

Previous researches demonstrated that the RSA and ELISA possess weak statistical agreement in *Mycoplasma gallisepticum* diagnosis.

These RSA and ELISA serological methods should be only used as screening tests in monitoring programs to detect avian mycoplasmosis in poultry flocks and positive results should be confirmed by routine microbiological tests. Differences in the results of the tests used in this study (RSA and ELISA) confirm this information, that the use of other techniques necessary to confirm the presence of the MG, such as culture and/or DNA detection by molecular assays (PCR).

Although the RSA and ELISA test was described to be less sensitive than the HI test, but it has the advantage of being rapid and easily performed and therefore can be utilized as a routine flock test.

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