COMPARATIVE EFFICACY OF RAPID SERUM AGGLUTINATION AND HAEMAGGLUTINATION INHIBITION TESTS IN THE SEROPREVALENCE STUDY OF MYCOPLASMA GALLISEPTICUM IN POULTRY BIRDS

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ABSTRACT: *The seroprevalence study of Mycoplasma gallisepticum (MG) in broiler, layer and breeder birds were designed to investigate the comparative efficacy of Rapid Serum Agglutination (RSA) and Haemagglutination Inhibition (HI) tests. A total of 435 serum samples were collected from 60 poultry farms, showing respiratory distress more than 50% of flock, for detection of MG infection. RSA were performed on collected sera samples showed the prevalence of MG in layer, broiler and breeder flocks as 67.2%, 60.5% and 68.2%, respectively. The sera positive for RSA were further tested by HI to quantify the antibodies. The results showed that the RSA test is more sensitive but less specific than HI test. For the rapid detection of MG, HI based assay is more sensitive, specific and reliable than conventional diagnostic techniques.*

Keywords: Seroprevalence; Mycoplasma gallisepticum; Rapid Serum Agglutination; Haemagglutination Inhibition

INTRODUCTION

Avian mycoplasmosis free rearing of chickens and turkeys, import of these birds, day old chicks and hatching eggs are of great concern with the forthcoming world trade organization strict recommendations [1]. Primarily, this is a disease of chicken and turkeys but also infects many other domestic and wild birds all over the world [2, 3].

It mainly caused by Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) that leads to considerable economic losses such as reduce weight gain, poor meat quality, increase in FCR and mortality in broiler, tremendous drop in egg production in layers and increases embryo mortality in breeders [4, 5, 6]. It is a cause of chronic respiratory disease (CRD), especially in the presence of management stresses and/or other respiratory pathogens. Losses attributed to mycoplasmosis are the decreased egg production, growth and hatchability rates [4] in layer and breeder. Production losses between 10-20% have been reported in layers [7].

All ages of chickens and turkeys are susceptible to this disease, but young birds are more prone to infection than adults [8]. The prevalence of MG was recorded highest at 20 weeks of age [9]. The prevalence of MG infection decreased with the increase of age in all flock according to individual flock area. Seasonal variation of prevalence of MG infection was also observed. The prevalence was higher (62.44%) in the winter season and lower (53.10%) in summer season [9]. The disease may be transmitted horizontally and vertically and remains in the flock constantly as subclinical form [10]. It has been shown that an association between infection of the oviduct with MS and the eggshell apex abnormalities (EAA) characterized by a roughened shell surface, shell thinning, increased translucency, cracks and breaks [11].

Shah [12] reported the serological evidence of avian mycoplasmosis in Pakistan. Mukhtar [13] has characterized MG and described its seroprevalence. Haque [14] has made isolation, identification and characterization of Mycoplasma species from respiratory problems in poultry. Investigation indicates that the pathogenicity of one strain (MI-211) was comparable with that of MG-S6, which is reported to be highly pathogenic. The early diagnosis of mycoplasma infections through molecular characterization along with isolation and identification of organisms are important considerations for a country like Pakistan to enhance the export of poultry and poultry products across the world.

Therefore, the present study was designed to evaluate the comparative efficacy of Rapid Serum Agglutination Test and Haemagglutination Inhibition Test in seroprevalence study of Mycoplasma gellisepticum in Pakistan.

MATERIAL AND METHODS

The present study was designed to evaluate the comparative efficacy of Rapid Serum Agglutination test and Haemagglutination Inhibition test to detect the Mycoplasma gallisepticum in invitro.

Samples Collection:

A Total of sixty farms (N=20 Breeder; N=20 Layer & N=20 Broiler) of commercially reared poultry birds, showing respiratory distress more than 50% of flock, were visited and the blood samples were collected for detection of Mycoplasma gallisepticum infection.

Preparation of Sera:

The total of 435 (Layer Flock=125; Broiler Flock=200 & Breeder Flock=110) blood samples were directly collected from the brachial vein of the suspected birds into a sterilized plastic bottles by using 5ml sterile disposable syringe. The blood samples were kept at room temperature and allowed to clot for the collection of sera [9]. After clotting, sera were separated, centrifuged and poured in sterile vials, labeled individually and stored at 4^{o}C until used. The collected sera samples were shifted to Mycoplasmology Laboratory of University of Agriculture, Faisalabad, Pakistan for further tests.

Diagnostic Tests:

i. **Rapid Serum Agglutination (RSA) Test:**

Seroprevalence of Mycoplasma gallisepticum was done with Rapid Serum Agglutination test. For this purpose, antigen was procured from the Mycoplasmology Laboratory of University of Agriculture, Faisalabad, Pakistan and Rapid Serum Agglutination test was carried out, as previously described by OIE [15], to determine the infection.

ii. Haemagglutination Inhibition (HI) Test:

The samples positive for the Rapid Serum Agglutination test were further tested by Haemagglutination Inhibition test to quantitate the serum antibodies. The positive samples were tittered by HI test, as described by the OIE [15], to standardize the values.

RESULTS AND DISCUSSION

1. Rapid Serum Agglutination Test

The overall seroprevalence of Mycoplasma gallisepticum in broiler, layer and breeder after Rapid Serum Agglutination test are shown in table 1. The results showed that the average percentage of prevalence of Mycoplasma gallisepticum in layer, broiler and breeder flocks were 67.2%, 60.5% and 68.2%; respectively. These findings are similar up to some extent with previously reported findings as highest prevalence 73.80% and the overall 58.9% seroprevalence of Mycoplasma gallisepticum in breeder poultry farms of Bangladesh [9]. The results also showed the seroprevalence of Mycoplasma gallisepticum in broiler and layer farms were 60.5% & 67.2%, which are in accordance with the previously reported findings as prevalence of Mycoplasma gallisepticum 66.50% in layer, in Bangladesh [16]. The results of this study also showed the 60.5 % seroprevalence of Mycoplasma gallisepticum in broiler which do not correlate with the previous findings as reported 49.50% prevalence [16]. This study showed the highest rate of seroprevalence of Mycoplasma gallisepticum. It was due to various Managemental factors in Pakistan. Dulali [17] also reported that the factors like poor ventilation, infection of litters, no restriction on movements of technical personnel and visitors contributes to Mycoplasma gallisepticum infection.

2. Haemagglutination Inhibition Test

The overall seroprevalence of Mycoplasma gallisepticum by Haemagglutination Inhibition test in broiler, layer and breeder are shown in table 2. The results showed that the prevalence of Mycoplasma gallisepticum in breeder flocks were higher by 61.2% as compared to layer and broiler flocks. Prevalence of Mycoplasma gallisepticum in layer birds was 57.9% followed by broiler (47.6%).

The Similar findings were also reported in 1975 by testing of 43,040 sera samples from 36 broiler flocks for antibodies to Mycoplasma gallisepticum by serum plate agglutination and Haemagglutination Inhibition tests [18]. They found a lower percentage of positive samples as compared to those much positive by the Rapid Serum Agglutination test. The same findings were also reported during this study. Previous studies reported that the Haemagglutination Inhibition test is more specific than Rapid Serum Agglutination test [18] which is in accordance to our results. Other researchers also reported some similar results in layers and in breeders [19] and [20], respectively. Ley [6] also reported that the serum plate agglutination test is more sensitive than that of ELISA and Haemagglutination Inhibition test. However, the serum plate agglutination test is more prone to false positive and nonspecific reactions [21, 22, 23, 24] as compared with others.

Table: 1 Seroprevalence of *Mycoplasma gallisepticum* in Poultry Birds by Rapid Serum Agglutination test

Flocks	Total serum sample	Positive cases	Prevalence (%)
Layer Flocks	125	84	67.2
Broiler Flocks	200	121	60.5
Breeder Flocks	110	75	68.2
TOTAL	**435**	**280**	**64.37**

Table: 2 Seroprevalence of *Mycoplasma gallisepticum* in Poultry Birds by Haemagglutination Inhibition test

Flocks	RSA + samples	%age of Haemagglutination Inhibition Titer			%age of positive samples
		<20	20-40	≥80	
Layer Flocks	84	42.1	36.9	21	57.9
Broiler Flocks	121	52.4	27.5	20.1	47.6
Breeder Flocks	75	38.8	36.7	24.5	61.2

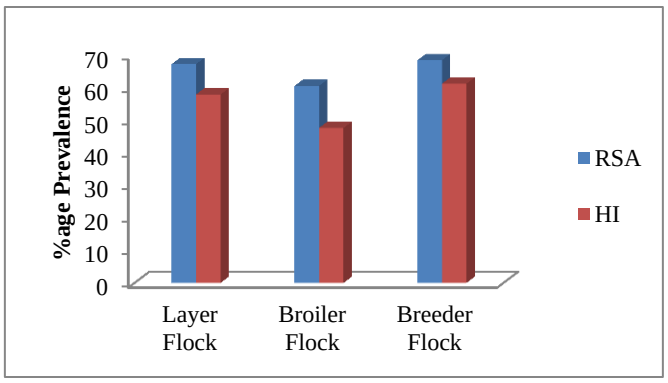

Figure 1: Percentage Prevalence of RSA and HI positive samples in Layer, Broiler and Breeder Flocks

Comparison of tests results for prevalence of Mycoplasma gallisepticum in poultry birds are shown in Fig. 1, which indicates that the highest prevalence of Mycoplasma gallisepticum was in breeder flocks followed by layer and broiler flocks, but the percentage of prevalence varies from one test to the other which indicating that one test is more sensitive than the other and vice versa.

CONCLUSION

On the basis of the results it concluded that the Rapid Serum Agglutination (RSA) test is highly sensitive for the detection of Mycoplasma gallisepticum in poultry birds but Haemagglutination Inhibition (HI) based assay is more specific and reliable than RSA and other conventional diagnostic techniques.

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