PREVALENCE OF *MYCOPLASMA SYNOVIAE* IN EGGS FROM LAYING HENS USING ELISA

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Summary

Mycoplasma synoviae (*M. synoviae*) can cause respiratory disease, synovitis, peritonitis, egg apical abnormalities or a subclinical infection. The importance of *M. synoviae* is well established in broilers but only a few studies have been conducted in layers. In the present study, the prevalence of *M. synoviae* in commercial layer flocks was determined by ELISA using egg yolk antibodies. Subsequently, a possible correlation between the serological status of *M. synoviae* and egg shell quality was also studied. In the flocks under study, seroprevalence of *M. synoviae* was found to be 69 % (95 % confidence interval (CI) = 47 to 91). Statistical analysis showed that the vaccinated group (3.0 ± 0.1) had the highest translucency score as compared to infected (2.4 ± 0.1) and uninfected (2.5 ± 0.1) groups, whereas % shell reflectivity was highest in the infected group (31.41 ± 0.3) as compared to the other two groups. Shell breaking strength (39.5 ± 0.5 Newtons) and shell deformation (298.7 ± 3.8 μm) values were significantly lower in the infected group than in the uninfected and vaccinated groups. There was no significant difference among these three groups for egg quality parameters egg weight, egg shell weight, % egg shell, shell thickness.

I.   INTRODUCTION

Mycoplasma synoviae (*M. synoviae*) can cause respiratory disease, synovitis, peritonitis, egg apical abnormalities or a subclinical infection (Feberwee et al. 2009a,b). Mycoplasma species are well-known pathogens of domestic poultry, causing significant economic losses (Lierz et al., 2007). The importance of *M. synoviae* is well established in broilers but only a few studies have been conducted in layers (Hagan et al., 2004). *M. synoviae* is known to be transmitted vertically through eggs (Board and Fuller, 1994). The prevalence of egg *M. synoviae* antibody in egg yolk has been found to be a suitable approach to assess the flock prevalence of *M. synoviae* infection in layer hens (Hagan et al., 2004) and found comparable with serum antibodies (Mohammed *et al*., 1986 a,b). Earlier, the Dutch strain of *M. synoviae* was found to be one of the factors causing egg shell translucency (Feberwee et al., 2009a,b). However, there is little information available regarding the effects of Australian strains of *M. synoviae* on egg shell quality. In the present study, the prevalence of *M. synoviae* in commercial layer flocks was studied by ELISA using egg yolk antibodies. Finally, correlations between egg shell quality parameters and the presence of *M. synoviae* in eggs was investigated.

II.   MATERIALS AND METHODS

Eggs were randomly collected from 19 different commercial layer flocks. Of these 19 flocks, three flocks were vaccinated. In general, 30 eggs from each flock were used for seroprevalence studies and 30 eggs from each flock were collected for determining egg quality parameters such as translucency score, shell reflectivity, egg weight, shell deformation, shell weight, % shell and shell thickness.

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The method for extracting antibodies from egg yolk was adapted from Mohammed et al. (1986b). For a saline extraction, 3 mL of egg yolk was collected from each of the 570 eggs (n=30 from 19 flocks) and mixed with 3 mL saline, vortexed and left for 48 h at 4°C. For the chloroform extraction, 0.5 mL saline extraction and 1 mL chloroform were vortexed to a thick paste. This was allowed to stand for 30 min at room temperature before being centrifuged at 850 x g for 20 min. The upper aqueous layer was removed and used in the ELISA. These extracted yolk antibodies were stored at -20°C. Each extracted antibody sample was diluted 1:50 ratio in PBS and was then used in the ELISA. The BioChek *Mycoplasma synoviae* antibody kits (BioChek B.V., Holland) were used in this study according to the manufacturer's instructions in order to study the prevalence of *M. synoviae* in the sampled commercial layer flocks. Absorbance of controls and test samples was recorded at 405 nm. Dilutions of chloroform-extract egg yolk antibody were prepared from the pools of known positive (*M. synoviae* vaccinated) and known negative eggs and tested for the following titres; 1:10, 1:50, 1:100, 1:500 and 1:1000. From the curve produced, the linear part was expanded. Reading the known positive and negative samples individually at the selected dilution produced a cut-off point for the test. The cut off values were determined using the model described by Greiner et al. (1995). The results were used to calculate the optimum sensitivity (Se) and specificity (Sp). The Se and Sp for each threshold value were calculated as the proportion of positive results in the positive reference population and negative results in the negative reference population, respectively (Greiner et al., 1995). Depending upon ELISA results, the flocks were divided into three groups: infected, uninfected and vaccinated. The flocks with more than 10% positive reactions were considered positive serologically based on the method of Kleven and Bradbury (2008). Using the ELISA results, seroprevalence of *M. synoviae* was determined at 95% confidence with a Binomial exact confidence interval model. ANOVA of the S-PLUS statistical software was used to compare egg shell quality parameters of infected, uninfected and vaccinated group.

III.   RESULTS

Using the chloroform-extracted egg yolks, a dilution factor of 1:50 was chosen as it was on the linear part of the standard curve produced. It was observed that the optimized optical density cut-off point was 0.390 with 90 % Se and Sp. Out of the 19 flocks screened under this study, numbers of serologically positive (infected) and negative (uninfected) flocks were found to be 11 and 5, respectively, and the remaining 3 flocks were vaccinated. Thus, the prevalence *of M. synoviae* serologically positive flocks in commercial layers was high {11/16 (69 %) , 95 % CI = 47 to 91}. Table 1 shows the individual flock-wise seroprevalence of *M. Synoviae*. Table 2 shows the relationship between *M. synoviae* serological status and different egg shell quality parameters (Translucency, shell reflectivity, egg weight, shell breaking strength, shell deformation, egg shell weight, % egg shell and shell thickness). Statistical analysis showed that the vaccinated group (3.0 ± 0.1) had the highest translucency score as compared to infected (2.4 ± 0.1) and uninfected (2.50 ± 0.1) groups whereas the infected group (31.4 ± 0.3) had the highest % shell reflectivity as compared to the other two groups. Shell breaking strength (39.5 ± 0.5 N) and shell deformation (298.7 ± 3.8 μm) values were significantly lower in the infected group than in the uninfected and vaccinated group.s However, there was no significant difference among the three groups for other egg quality parameters such egg weight, egg shell weight, % egg shell, shell thickness.

Table 1. Individual flockwise seroprevalence of *M. synoviae* in unvaccinated flocks

Flock number	Sample size	*M. synoviae* serologically positive	Prevalence (95% CI) of seropositive farms (%)
1	30	11	37 (20 to 54)
2	30	15	50 (32 to 68)
3	30	12	40 (22 to 58)
4	30	20	66 (49 to 83)
5	30	0	0
6	30	1	3 (0 to 9)
7	30	29	97 (91 to 100)
8	30	4	13 (1 to 25)
9	30	8	27 (11 to 43)
10	30	3	10 (3 to 26)
11	30	1	3 (0 to 9)
12	30	11	37 (20 to 54)
13	19	12	63 (41 to 85)
14	30	10	33 (16 to 50)
15	30	0	0
16	30	0	0

Table 2.   Relationship between *M. synoviae* serological status and different egg shell quality parameters

Variables	Infected	Uninfected	Vaccinated
Number of Flocks	11	5	3
Translucency	$2.4^a \pm 0.1$	$2.5^a \pm 0.1$	$3.0^b \pm 0.1$
% Shell reflection	$31.4^a \pm 0.3$	$28.7^b \pm 0.5$	$29.7^b \pm 0.5$
Egg weight (gm)	$61.5^a \pm 0.4$	$60.2^a \pm 0.9$	$59.9^a \pm 0.5$
Shell breaking strength (N)	$39.5^a \pm 0.5$	$43.2^b \pm 1.0$	$45.9^b \pm 1.3$
Shell deformation (μm)	$298.7^a \pm 3.8$	$321.9^b \pm 10.1$	$310.5^b \pm 8.8$
Egg shell weight (gm)	$5.8^a \pm 0.1$	$5.69^a \pm 0.1$	$5.6^a \pm 0.1$
% Egg shell	$9.4^a \pm 0.1$	$9.5^a \pm 0.1$	$9.3^a \pm 0.1$
Shell thickness (μm)	$390.9^a \pm 2.5$	$385.6^a \pm 3.1$	$383.9^a \pm 3.4$

Means with the different superscript in the same row are statistically significantly different ($P \geq 0.05$) from each other.

IV.   DISCUSSION

The present study was conducted in order to determine the seroprevalence of *M. synoviae* infection in commercial layer flocks by ELISA. A high seroprevalence of *M. synoviae* in commercial layer flocks was found. This finding is in agreement with data of other research groups. The study of Hagan et al. (2004), which was also based on the detection of *M. synoviae* antibodies in eggs, reported a prevalence of 78.6% in commercial layer flocks in East England. In another study (Mohammed et al., 1986), a *M. synoviae* prevalence of 87% was found in commercial layer flocks in Southern California. In the present study, sample size is relatively small compared to earlier studies and study is ongoing. The high prevalence and persistence of *M. synoviae* infections in layer stock have been explained by the frequent occurrence of multiple-age housing and lower biosecurity standards in this sector (Stipkovits & Kempf, 1996; Kleven, 2003). *M. synoviae*-infected commercial layer stocks therefore pose a significant epidemiological risk for other categories of poultry. Feberwee et al. (2009a,b) reported that a Dutch strain of *M. synoviae* was associated with formation of egg apex abnormalities (EAA) and also reported synergism between *M. synoviae* and infectious bronchitis virus. In the present study, it was found that shell breaking strength and shell deformation were significantly lower in the infected group as compared to the uninfected and

vaccinated groups. The vaccinated group had the highest translucency score as compared to infected and uninfected groups, whereas the infected group had lighter coloured shells as compared to the other two groups. Findings of the study are in contrast to earlier findings by Lott et al. (1978) who found that *M. synoviae* infection in broiler breeders had no effect on egg shell strength or Haugh units under experimental conditions. These differences in findings might be because the present study was conducted in field conditions. However, controlled experiments would be necessary to study the effects of the Australian strains of M. *synoviae* on egg quality of commercial layers.

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