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Evaluation of an egg yolk enzyme-linked immunosorbent assay antibody test and its use to assess the prevalence of *Mycoplasma gallisepticum* infection in laying hens in Italy

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ABSTRACT

The prevalence of *Mycoplasma gallisepticum* infection in commercial layers was established by the presence of antibodies in eggs. Saline-extracted yolks were used with a commercial enzyme-linked immunosorbent assay kit. For the prevalence study, yolks from 30 eggs were obtained from each of 66 flocks coming from 36 layer farms. The prevalence of egg antibodies to *Mycoplasma gallisepticum* was 33.3% in single-age farms and 77.8% in multi-age farms. In 27 flocks, antibody titers were compared with results obtained from blood samples taken in the same flock and in the same period and analyzed with the same kit. This study has confirmed that egg yolk enzyme-linked immunosorbent assay antibody test is a suitable and practical approach for assessing the flock prevalence of *Mycoplasma gallisepticum* infection in layer hens.

Key Words: Layer hens, *Mycoplasma gallisepticum*, ELISA test, Egg yolk.

RIASSUNTO

APPLICAZIONE DI UN TEST IMMUNOENZIMATICO PER LA DETERMINAZIONE DEGLI ANTICORPI NEL TUORLO E SUO IMPIEGO NELLA VALUTAZIONE DELLA PREVALENZA DELL'INFEZIONE DA *MYCOPLASMA GALLISEPTICUM* NEGLI ALLEVAMENTI DI GALLINE OVAIOLE DA CONSUMO IN ITALIA

La prevalenza dell'infezione da *Mycoplasma gallisepticum* in allevamenti di galline ovaiole da consumo è stata determinata attraverso la valutazione del titolo anticorpale nel tuorlo (dopo estrazione in soluzione salina) mediante un test immunoenzimatico commerciale. Per lo studio di prevalenza sono stati esaminati campioni di 30 uova provenienti ciascuno da 66 gruppi di galline ovaiole appartenenti a 36 aziende. In 27 gruppi i titoli anticorpali rilevati nel tuorlo sono stati confrontati con quelli riscontrati in analoghe campionature di sangue. La prevalenza di anticorpi nel tuorlo nei confronti di *Mycoplasma gallisepticum* è stata del 33,3% in allevamenti costituiti da un'unica unità produttiva e del 77,8% in allevamenti multi-età. Lo studio ha inoltre confermato che la determinazione del titolo anticorpale nel tuorlo nei confronti di *Mycoplasma gallisepticum* fornisce risultati sovrapponibili a quelli ottenuti dalla medesima ricerca eseguita in campioni di sangue. Rispetto a quest'ultima, inoltre, fornisce alcuni vantaggi di ordine pratico.

Parole chiave: Galline ovaiole, *Mycoplasma gallisepticum*, Test ELISA, tuorlo.

Introduction

Mycoplasma gallisepticum (MG) is an important pathogen of poultry worldwide. MG infection is considered an important problem in broilers, breeders and commercial layers. Economic losses in the poultry industry caused by this infection are

significant. In breeders and layers the disease causes a 10% to 20% decrease in egg production (nearly fewer 16 eggs per hen) and a 5% to 10% increase in embryo mortality. In addition MG cause respiratory disease commonly complicated by other agents such as *Escherichia coli*. The mortality can be low in uncomplicated disease but may reach 15-

20% in complicated outbreaks. Few data are available about the prevalence of MG infection in commercial layers in Italy. In routine work detection of MG infection may be achieved by detecting antibodies against MG in the host organism. For this purpose various tests are used including serum plate agglutination (SPA), haemagglutination-inhibition (HI) and enzyme-linked immunosorbent assay (ELISA). Several ELISA kits are available. This test is more specific than SPA and more sensitive than HI. In addition, ELISA test is used for testing of yolk samples.

The aims of this study were the following: 1) to evaluate a commercial ELISA kit when used to measure MG antibodies in eggs. 2) To study the prevalence of MG infection in layer farms located in different parts of Italy.

Material and methods

ELISA test

The ProFLOK® MG ELISA kit (Synbiotics corporation®) was used in this study according to the manufacturer's instructions. The absorbance was read at 405 nm on a Biorad 550 microplate reader (BIORAD®). In order to be valid the mean negative control absorbance should be below 0.200 and the corrected positive control value range should be between 0.250 and 0.900. The serum/positive ratio (SP) was calculated as: (mean of test sample – mean of negative control) / (mean of positive control – mean of negative control). An MG titer was calculated by the following suggested equation: $\text{Log}_{10} \text{ titer} = (1.464 \times \text{Log}_{10} \text{ SP}) + 3.197$. Yolk samples were pre-diluted (1:10) using phosphate buffer solution (PBS). According to the manufacturer's

instructions the SP and the ELISA titer was interpreted using the following value ranges: SP less than 0.200 (titer range 0) = negative; SP 0.200 to 0.599 (titer range 149 to 743) = probable or not conclusively; SP greater or equal to 0.600 (titer 744 or greater) = positive. A flock was considered positive when antibody titer was greater than 744 in more than 10% of the tested samples. In multi-age farms, a farm was considered positive when one or more production unit (flock) resulted positive.

Prevalence study

Thirty eggs/flock were collected. In some flocks blood samples were also obtained. For every flock, anamnestic data including age, production type (cage or floor farms), location of the farm, number of animal housed and clinical signs were registered.

Statistical analysis

The geometric mean titers and the standard deviations for every sample of yolk and blood were calculated. Comparison between serum and yolk titers were made by a *t*-test.

Results and discussion

Prevalence study

During the period November 2003 – January 2004, 36 commercial layer farms located in 7 different Italian regions were monitored (Table 1). Overall, eggs from 66 flocks were tested. In 27 flocks blood samples were also examined. Approximately 2,200,000 of housed layers were involved in this study. 33 of 66 flocks tested resulted positive. The correlation between MG antibody status of the flock and the observation of clinical

Table 1. Geographical distribution of tested farms in Italy.

Region	Single-age farms	Multi-age farms	Total
Emilia Romagna	5	11	16
Veneto	3	5	8
Lombardia	5	1	6
Marche	3	0	3
Toscana	0	1	1
Piemonte	1	0	1
Campania	1	0	1

Table 2. Correlation between MG antibody *status* and clinical signs.

MG antibody status / clinical signs	n. flocks
MG+ / clinical signs +	14
MG+ / clinical signs -	19
MG- / clinical signs +	8
MG- / clinical signs -	25
Total	66

signs related to MG infection (respiratory signs and/or drop in egg production and/or decreased egg shell quality) is summarized in table 2. 14 of 18 multi-age farms resulted positive for MG antibodies giving a prevalence of 77.8%. In single-age farms the prevalence was 33.3%.

Comparison between yolk and serum titers

Comparison between blood and yolk titers were made in 27 flocks. Descriptive statistics are reported in table 3. For all tested flocks no significant differences in level of antibodies were detected between antibody titers in layer serum and these obtained in egg yolk ($t=0.947$; $d.f.=1013$; $p=0.344$) (Figure 1).

Conclusions

The findings from the prevalence study indicated that MG infection was higher in multi-age farms. MG infection persists in the flock indefinitely and the chickens may shed the organism intermittently, especially following a period of stress. As MG free pullets are brought onto the multi-age complex, they are exposed to MG infection at the onset of egg production. This cycle of spread con-

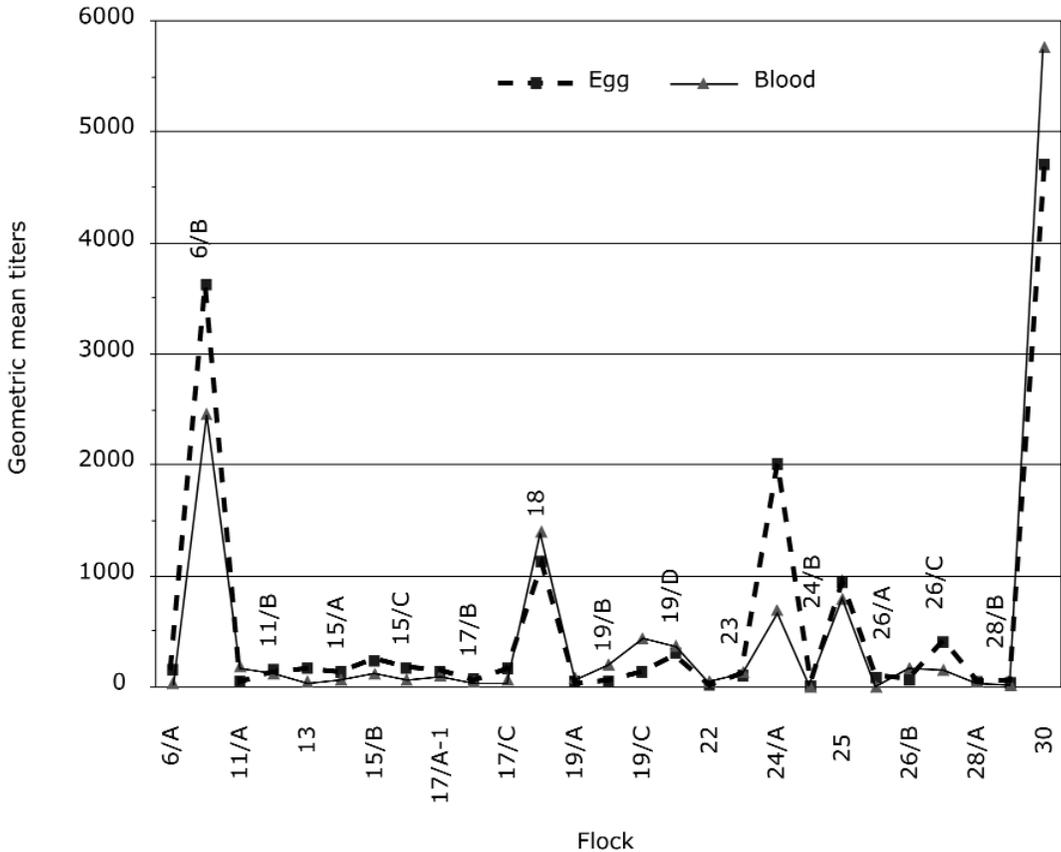
tinues in a complex with new flock introductions (Levisohn *et al.*, 2000). In many cases detection of MG antibodies were not associated to clinical signs. A possible explanation could be the variability of clinical symptoms of MG between species. Infected adult chickens may show no outward signs if the infection is uncomplicated (Stipkovits *et al.*, 1996).

Previous studies indicates that chloroform extraction of the egg yolks is the more suitable method to obtain a greater distinction between positive and negative samples (Mohammed *et al.*, 1986; Hagan *et al.*, 2004). A possible explanation of the benefits of this method of extraction is that chloroform removes the lipids from the samples that otherwise interfere with the binding of antibodies to the antigen. However in our study no significant differences were observed between antibody titers obtained in blood samples e these detected in egg yolk after saline extraction. Testing eggs for routine screening of MG infection avoids the expense of blood sampling, the need for trained staff and subjecting the birds to the stress of being handled. In addition, sampling personnel can spread infection from farm to farm. Finally, eggs are easy to collect and inexpensive.

Table 3. Descriptive statistics.

Statistical data	Egg yolk	Blood
Number of tested samples	810	205
Geometric mean	156.8	137.3
Arithmetic mean	664.1	734.7
Median	132	115
SD	1310.9	1613.9
Min.	1	1
Max.	7762	9333

Figure 1. Comparison between geometric mean titers obtained in blood and in egg yolk samples.



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