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## Therapeutic Efficacy of Chicken Egg Yolk Immunoglobulins Against *Mycoplasma gallisepticum* Infection in Chickens

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**Abstract:** The focus of this report was to assess the role of passive immunization using immunoglobulins derived from chicken egg yolk in protection against *M. gallisepticum* infection in chickens. Immunoglobulins were produced by egg yolk technology. ELISA was used to evaluate their immunoreactivity and western immunoblotting demonstrated that chicken egg yolk immunoglobulins Y (IgY) were capable of recognizing *M. gallisepticum* immunoreactive antigens. Administration of IgY to experimentally infected broiler chickens afforded up to 70% protection rate as confirmed by culture analyses. Challenge of IgY - treated birds with *M. gallisepticum* virulent strain S6 revealed significantly ( $P < 0.05$ ) lower isolation prevalence in comparison to control chickens treated with IgY from mock-immunized (control) hens that received the same bacterial challenge. Additionally, *M. gallisepticum* culture analysis from respiratory organs demonstrated that these antibodies protected chickens in a dose-dependent pattern against *M. gallisepticum* infection (i.e., increasing IgY titer resulted in decreased isolation rate). These findings demonstrate that IgY obtained from hens immunized by *M. gallisepticum* could provide an alternative approach for treatment of *M. gallisepticum* infection in chickens.

**Key words:** *Mycoplasma gallisepticum*, egg-yolk antibodies, immunoglobulin therapy, passive antibody transfer

### Introduction

*Mycoplasma gallisepticum* remains the most frequently reported bacterial causative agent of chronic respiratory disease in chickens and infectious sinusitis in turkeys (Yoder, 1991; Ley and Yoder, 1997). In addition to condemnation of the infected flocks *M. gallisepticum* infection causes reduced feed conversion and egg production, resulting in serious economic losses (Ley and Yoder, 1997; Levisohn and Kleven, 2000). Therefore, protection against *M. gallisepticum* infection is of great interest to the commercial poultry breeders. Traditionally, control of *M. gallisepticum* has been based on eradication of the microbe from breeder flocks and on maintenance of the mycoplasma-free status in the breeders and breeder progeny by biosecurity of the premises (Levisohn and Kleven, 2000). Efforts to reduce the adverse affects of the disease on breeders in complexes also included the use of antibiotics and vaccines. Although useful, these efforts have not been successful in eliminating infection and shed (Kleven, 1986; Yagihashi *et al.*, 1986; Noormohammadi *et al.*, 2002; Reinhardt *et al.*, 2005).

Tremendous efforts have been done to evaluate numerous therapeutic regimens for eradication of *M. gallisepticum* infections. However, the continued presence and increasing incidence of *M. gallisepticum*

in commercial poultry farms suggests that efforts at eradication were not highly successful. Indeed, *M. gallisepticum* has made a staggering comeback for a variety of reasons, including reemergence of *M. gallisepticum* outbreaks among poultry due to the practice of placing large poultry populations in small geographical areas under poor sanitary conditions, the increased use of live *M. gallisepticum* vaccines, the detection of *M. gallisepticum* in game and free-flying songbirds, and development of resistance against some existing antibiotics (Kleven, 1977, 2003). As a consequence, *M. gallisepticum* infection in the poultry industry will likely continue and limiting losses will be the primary goal. Hence, it is important to seek new, safe and effective therapies for a wider means of treating, suppressing, or preventing *M. gallisepticum* infection. Passive immunization via the administration of pathogen specific antibodies to the bird prior to or after exposure to the organism is one of the alternative strategies that were almost entirely over looked with the introduction of antibiotics. The use of egg yolk antibodies (immunoglobulin Y (IgY)) in biomedical research is now gaining interest again due to many advantages associated with IgY (Kovacs-Nolan and Mine, 2004; Schuijffel *et al.*, 2005). Passive immunization with IgY has been used to prevent a variety of infections such as

those caused by *Salmonella enterica* serovar *Typhimurium* (Sunwoo *et al.*, 1996), enterotoxigenic *Escherichia coli* (Marquardt *et al.*, 1999), rotavirus (Sarker *et al.*, 2001), and Birnavirus (Malik *et al.*, 2006). However, despite being a convenient source of antibodies, there has been no report so far on the use of IgY in the prevention and treatment of *M. gallisepticum* infection.

In this report, the therapeutic activity of IgY against *M. gallisepticum* infection in chicken was investigated. The study demonstrated that IgY prepared from egg yolk of hens immunized with *M. gallisepticum* is effective in the treatment of *M. gallisepticum* infections. Unraveling of the protective immunity acquired during *M. gallisepticum* infection may contribute to development of effective control strategies.

## Materials and Methods

**Growth and maintenance of *Mycoplasma* strain:** *Mycoplasma gallisepticum* S6 strain and F strain live cultures were obtained from the depository at the Division of *Mycoplasma*, Animal Health Research Institute, Dokki, Giza, Egypt. *M. gallisepticum* strains were cultivated in Bacto-PPLO *Mycoplasma* broth or agar (Difco Laboratories) supplemented with 5% Yeast extract, 2% Thallium acetate, 100000 IU Penicillin G sodium, 15% Horse serum at 37°C. Logarithmic-phase cultures of *M. gallisepticum* strain S6 that contained approximately  $3 \times 10^7$  CFU/ml were used in challenging vaccinated animals.

**Animals:** *Mycoplasma*-free white leghorn broiler (line: Hyline strain W36) chickens (*Gallus gallus*) were obtained from a local breeder and housed in disinfected cages under controlled hygienic measures. They were fed on nonmedicated food and water was provided *ad libitum*. For monitoring of chickens prior to infection or chickens in the noninfected control group, tracheal swab samples were inoculated in broth by standard methods for the isolation of avian mycoplasmas (Ley and Yoder, 1997; Levisohn and Kleven, 2000). *Mycoplasma*-free chickens were only included in this study. The Institutional Animal Care and Use Committee approved all described procedures.

**Immunization procedure:** For the preparation of chicken egg yolk anti-*M. gallisepticum* F strain Ig, five-month-old chickens were used. Chickens were divided into two groups: first group were immunized intramuscularly in the breast muscle. Each bird was injected with 1 ml ( $1 \times 10^5$  colony forming unit (CFU)) of *M. gallisepticum* F strain live culture emulsified in an equal volume of Freund's complete adjuvant (Difco Laboratories). This group was used for the induction of specific antibodies in egg yolk. The second group of chickens was injected with Freund's complete adjuvant alone and was used as

control immunogen. Six weeks later, chickens were received one subsequent booster injection with the same antigen concentration in Freund's incomplete adjuvant (1: 1). One week later after the final booster, eggs were collected daily for up to 1 month and stored at 4°C until antibody extraction.

**Isolation and purification of IgY:** Isolation of IgY was carried out by the dextran sulphate precipitation method as described (Akita and Nakai, 1993). Briefly, egg yolk was carefully separated from the egg white and the yolk membrane with forceps. The yolk preparation was mixed with 4 volumes of Tris-buffered saline (TBS). The mixture was centrifuged at 1,500g for 20 min at room temperature (R.T.). A 120- $\mu$ l of dextran sulphate solution per ml of sediment were added, mixed well and incubated at R.T. for 30 min. A 50- $\mu$ l of 1M  $\text{CaCl}_2$  was added, mixed and incubated for further 30 min. The mixture was centrifuged in a CR/CT4.12 centrifuge with a M4 swinging bucket rotor (Forma Scientific, Marietta, OH) at 1,500g for 30 min at 4°C. After centrifugation, the pellet was resuspended in 2 ml sodium phosphate buffer. Separated egg yolk was pooled and stored at -20°C prior to IgY purification.

Lipid-free yolk was stirred mixed slowly with 20 g  $\text{Na}_2\text{SO}_4$ . Then, the solution was let to stand for 30 min and centrifuged at 5000 rpm for 20 min. Supernatant was discarded and the sediment was resuspended in 10 ml TBS. The solution was then centrifuged at 5000 rpm for 20 min and the supernatant was stirred and 8 ml 36% (W/V)  $\text{Na}_2\text{SO}_4$  was added and the solution was let to stand for 30 min and centrifuged as before. The supernatant was discarded and the sediment was resuspended in 5 ml TBS buffer. The sediment, which contains the water-soluble immunoglobulin rich fraction was collected and stored at -20°C until use.

**Titration of antibodies by ELISA:** To assess the IgY titer against the challenge strain of *M. gallisepticum*, the enzyme-linked immunosorbent assay (ELISA) was performed. *M. gallisepticum* whole-cell lysate antigens were prepared by ultrasonication of broth-cultivated organisms. Briefly, *M. gallisepticum* F strain was harvested by centrifugation at 1,200g for 10 min, suspended in phosphate-buffered saline solution (PBSS, pH 7.0) and disrupted by sonication. Cellular material was removed by centrifugation and the supernatant was collected (*M. gallisepticum* whole-cell lysate). Flat-bottomed ninety-six-well plates (Flow Laboratories, McLean, Va., USA) were coated with a 100  $\mu$ l of solution of the *M. gallisepticum* whole-cell lysate antigen (50  $\mu$ g/well) in 0.05M carbonate buffer (pH 9.6) at R.T. overnight. The plate was washed three times using PBS-Tween 20, then the free binding sites were blocked by 200  $\mu$ l/well of 0.2% nonfat powdered milk in coating buffer for 1 h at R.T. and then, plate was washed

three times with PBS-T20. After blocking of the wells, 50 µl of egg yolk antibodies were added to the wells using eight serial two-fold dilutions, starting at 1000 µg/ml and incubated at 37°C for 3 h; the plate was then washed as mentioned before. A 50-µl of alkaline phosphatase conjugated goat anti-chicken IgY (Sigma-Aldrich, MO, USA) at dilution of 1:500 in conjugate buffer was added to each well. The plate was incubated for 1 h at 37°C. For development of the color reaction the plates were incubated with 50 µl/well of freshly prepared P-NPP in substrate buffer at 37°C. After incubation for 30 min, the reaction was stopped by addition of 25 µl/well stopper solution (3 M NaOH). The absorbance was measured at 405 nm using a microplate reader (Bio TEK, VT, USA). Controls for this assay comprised sera from *M. gallisepticum* infected chickens as positive control and from non-infected chickens as negative control. Test groups included sera from passively immunized chickens against experimental *M. gallisepticum* infection. Experiment was conducted in triplicates.

**Electrophoresis and western immunoblotting:** In order to determine the specificity of produced egg yolk antibodies against *M. gallisepticum*, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed. Whole-cell preparations of *M. gallisepticum* S6 strain used in this study was suspended at a protein concentration of 1mg/ml in SDS-PAGE lysis buffer (50 mM Tris-HCl (pH 6.8), 2% SDS, 10% (vol/vol), 2% beta-mercaptoethanol, 0.1% bromophenol blue), heated in a boiling water bath for 3 min, and stored at -20°C until used. *Mycoplasma* proteins were separated by SDS-10% PAGE and the gel was electrophoretically transferred to nitrocellulose membrane filters (0.45-µm-pore size; Sigma-Aldrich, USA) for immunoblotting as described (Tsang *et al.*, 1983). Western blot was blocked with blocking buffer (5% w/v instant nonfat milk powder in PBS) and incubated for 1 h with the polyclonal anti-*M. gallisepticum* primary IgY in blocking buffer. The membranes were washed three times with TBS (pH 7.4) for 5 minutes and subsequently incubated with alkaline phosphatase-coupled rabbit anti-chicken antibodies for 2 h. For *M. gallisepticum* protein detection, the blots were then soaked in alkaline phosphatase substrate BCIP/NBT system. The color reaction was observed within 5 min and then the reaction was stopped after color formation by dipping the blots in distilled water. The blots were then air dried and kept in dark. Prestained SDS-PAGE standard (Bio-Rad Laboratories, USA) was used as molecular weight markers. All steps were carried out at R.T.

**Passive immunization with IgY against *M. gallisepticum* infection in chickens:** Eighty one-day-old broiler chickens from a *mycoplasma*-free local

commercial breeding flock were used to evaluate the protective efficacy of antibody to exposure to *M. gallisepticum*. Twenty chicks were used as negative controls for confirmation of *Mycoplasma*-free status. Chickens in this group were inoculated with sterile *Mycoplasma*-free broth medium. Additionally, chicks (n = 140) were infected with 100 µl containing  $3 \times 10^7$  CFU of *M. gallisepticum* S6 strain suspended in Difco PPLO broth. The challenge inoculum was inoculated into the posterior air sac of passively immunized birds by using a MicroAliquator (Scientific Manufacturing Industries). One day post infection, *M. gallisepticum* - infected chickens were randomly divided into seven experimental groups (n = 20/group) as follows: (i) *M. gallisepticum* infection only and served as a positive control, (ii) treatment with 7 µg of IgY /ml and (iii) treatment with 15 µg of IgY/ml, (iv) treatment with 31 µg of IgY/ml. Egg yolk antibodies were administered intraperitoneally daily for seven days (i.e., groups ii, iii and iv were injected 7 times with 1 ml solution containing 7, 15 and 31 µg IgY, respectively). 7, 15 and 31µg IgY were the highest egg yolk antibodies dilutions that had immunoreactivity to *M. gallisepticum* whole-cell lysate antigen compared to control antibodies. (v to vii) groups were administered an identical amount of the same three different concentrations of egg yolk antibodies but from adjuvant-immunized chickens (control immunogen). All groups were housed in separate rooms with absolute filters on in and outlet of ventilation to prevent infection from control chickens to IgY treated chicken.

**Evaluation of efficacy of immunization:** Evaluation of protective capacity of IgY was carried out in chicken challenged with live virulent strain S6 of *M. gallisepticum* followed by IgY administration seven times at 24 h intervals, with the first dose administered 24 h after infection; Birds were sacrificed one week after last immunization dose and swabs were taken from lung, trachea, and air sac. The colonization of *M. gallisepticum* in the respiratory organs in chickens was evaluated by culturing respiratory swabs taken at the time of necropsy of all chickens. All steps were carried out under sterile conditions including oropharyngeal swabs from living chickens. At each sampling time, tracheal swab samples were obtained from chickens for isolation and phenotypic analysis of *M. gallisepticum*. Swabs were cultivated in Bacto-PPLO *Mycoplasma* broth for enrichment. This is an essential step in cultivation from samples that contain a potential inhibitor (antibodies). Then, prevalence of *M. gallisepticum* was estimated by direct plating of inoculated broth culture onto *M. gallisepticum* selective agar and incubating the plates in a humidified incubator at 37°C in 5 % CO<sub>2</sub>/95% air. The agar surfaces were thereafter observed with an inverted microscope (Leitz) for the presence of colonies with a fried-egg appearance after 48 hours incubation and then

Every other day up to 10 days. The resulting colonies were identified as *M. gallisepticum* by microscopy and biochemical analysis of Glucose fermentation and Arginine deamination activities. Briefly, for glucose fermentation test, 2.9 ml of glucose medium was added to 0.1 ml of suspected *Mycoplasma* culture, incubated at 37°C and examined daily for 7 days before final conclusion was taken. Change of color from orange to yellow indicates positive result while no color change indicates negative results. For Arginine deamination test, 0.1 ml of the suspected viable *Mycoplasma* culture was inoculated into 2.9 ml of the medium and incubated aerobically at 37°C for 7 days, together with an uninoculated control tubes. Change of color from violet to dark red or reddish violet coloration indicates positive.

**Statistics:** Statistical analysis of the bacterial isolation in response to increasing IgY titer in the animal experiments employed the Kruskal-Wallis nonparametric analysis of variance (multi group comparison) with the Dunn procedure used for pairwise comparisons. Analysis was done with SAS software (version 8.02; SAS Institute, Cary, N.C.). P values of <0.05 were considered statistically significant. ELISA titers were calculated by linear regression plotting of optical density (OD) values versus the log<sub>10</sub> of the serum dilutions. The reciprocal of the calculated dilution giving a reading of 0.2 OD was arbitrarily defined as the endpoint titer.

## Results

**Immunoreactivity of IgY:** Our objective was to evaluate the potential efficacy of passive immunotherapy for chickens at risk with egg yolk-derived antibodies as the sole treatment regimen to prevent *M. gallisepticum* infections. To evaluate this strategy, polyclonal egg yolk-derived IgY antibodies to *M. gallisepticum* F strain were prepared. Then, immunologic analysis of antibody titers and determination of their immunoreactivity with *M. gallisepticum* antigens were carried out. First, IgY obtained from hens without and with *M. gallisepticum* immunization, respectively, was examined for immunological properties by ELISA. Evaluating antibody levels against the *M. gallisepticum* antigens confirmed the immunoreactivity of IgY. The ELISA readings for the highest IgY concentrations were 0.487 and 1.82 for IgY adjuvant and IgY immunized, respectively. These data demonstrated immunoreactivity between the F strain antigens and homologous antibodies and indicated that produced IgY is immunoreactive to *M. gallisepticum* (Fig. 1).

Secondly, immunoreactivity of IgY derived from chicken immunized with whole *M. gallisepticum* F strain was evaluated by western immunoblotting. SDS-PAGE showed differences on protein banding patterns when probed with polyclonal IgY derived from egg yolk of

chickens to F strain immunogen and egg yolk-derived antibodies of adjuvant-immunized chicken. Antibodies obtained from hens injected with adjuvant was used as a control and reacted with 74, 88, 94 and 96 kDa. On the other hand, IgY from immunized chicken recognized multiple bands with molecular sizes (M<sub>r</sub>) 31, 36, 39, 44, 74, 88, 94, and 96 kDa protein from a *M. gallisepticum* S6 strain lysate (Fig. 2). Additionally, bands 74, 88, 94 and 96 kDa were more distinct when treated with polyclonal IgY from immunized chicken compared to those treated with polyclonal IgY of adjuvant-immunized chicken. These data demonstrated strong cross reactivity between the S6 strain antigens and heterologous F strain antibodies. These antibodies recognized most peptide bands from heterologous strain S6. Also, the IgY was a valuable tool for the identification of immunogenic antigens of *M. gallisepticum*.

**Passive immunotherapy studies:** The growth inhibition activities against *M. gallisepticum* S6 strain by the IgY are shown in Table 1. In groups of chicken treated with egg yolk-derived antibodies from adjuvant-immunized chicken, S6 infection resulted in high levels of bacterial isolation in respiratory organs. In contrast, IgY-immunized chicken groups were protected against challenge with the heterologous S6 strain. Passive transfer of these IgY antibodies to these chickens significantly (P<0.001) produced lower bacterial isolation in the same organs. Antibodies to *M. gallisepticum* F strain antigens were effective as an immuno therapeutic agent in chickens when passive therapy was initiated 24 h after live S6 bacterial challenge. Anti-*M. gallisepticum* egg yolk antibodies at three different concentrations as determined by ELISA conferred dose-dependent protection against infection with *M. gallisepticum* heterologous strain S6. A high dose (15 and 31 µg/ml) of IgY treatment decreased *M. gallisepticum*-isolation from respiratory organs' swaps. However, the group that was administered lower dose (7 µg/ml) of IgY failed to protect chickens from *M. gallisepticum* infection and showed statistically non-significant difference with the *M. gallisepticum* infection group (P<0.3). With increased IgY titer, isolation rates decreased; birds immunized with 7 µg/ml had a prevalence of 80% positive, 53.3% of chickens immunized with 15 µg/ml birds were positive, and 33.3% of birds immunized with 31 µg/ml birds were positive for *M. gallisepticum*.

Also, data in Table 1 shows that isolation rates varied with the immunization status; with immunized chicken, the prevalence ranged from 30 - 90%. In the adjuvant-immunized chicken the prevalence was = 70% and no significant differences were noted for the protective effect of the different concentrations of control antibodies that do not contain specific antibodies. No mortality was recorded in chicken of all tested groups till the end of the study. However, all chickens from which bacteria were

Table 1: Rates of *M. gallisepticum* isolation from respiratory organs of chickens experimentally infected with S6 strain of *M. gallisepticum* and passively immunized with anti-*M. gallisepticum* F strain egg yolk antibodies at various titers

Group	Antibodies (IgY) (Dose and type)	No. of swabs positive/total (%)		
		Trachea	Lung	Air sac
G1	None	20/20 (100)	16/20 (80)	20/20 (100)
G2	7 µg/ml, immunized	18/20 (90)	14/20 (70)	16/20 (80)
G3	15 µg/ml, immunized	12/20 (60) <sup>A</sup>	10/20 (50) <sup>A</sup>	10/20 (50) <sup>A</sup>
G4	31 µg/ml, immunized	8/20 (40) <sup>A</sup>	6/20 (30) <sup>A</sup>	6/20 (30) <sup>A</sup>
G5	7 µg/ml, adjuvant	18/20 (90)	16/20 (80)	16/20 (80)
G6	15 µg/ml, adjuvant	14/20 (70)	16/20 (80)	16/20 (80)
G7	31 µg/ml, adjuvant	14/20 (70)	14/20 (70)	16/20 (80)

A Statistically significant differences from the values for the *M. gallisepticum* infection group 1 ( $P < 0.05$ ).

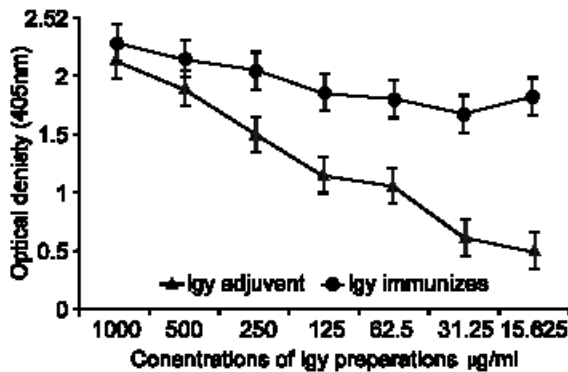


Fig. 1: The reaction between IgY and *M. gallisepticum*. 50 microliters of IgY was added with a two fold serial dilution in 96-well plates coated with *M. gallisepticum* whole-cell lysate (500 ng/well), and the titers were measured using ELISA. IgY was isolated from the egg yolk of immunized hens (●) and IgY was obtained from hens immunized with adjuvant only (▲). Each point represents mean  $\pm$  SD from three independent experiments.

isolated were also found to exhibit clinical respiratory manifestations. Severity of clinical signs decreased with increased IgY titer. All *M. gallisepticum* S6 strain isolated from chickens was glucose positive and arginine negative.

## Discussion

Development of effective immunization strategies necessitates knowledge if antibodies can confer protection against *M. gallisepticum*. The main hypothesis tested in this study was that *M. gallisepticum*-specific egg yolk-derived immunoglobulins (IgY) are efficient therapeutic agent against *M. gallisepticum* infection. Chickens were immunized with F strain of *M. gallisepticum* to produce IgY for protection trials against challenge with the virulent S6 strain of *M. gallisepticum* in chickens. ELISA and western blotting analysis demonstrated a pronounced specificity of IgY against *M. gallisepticum* antigens.

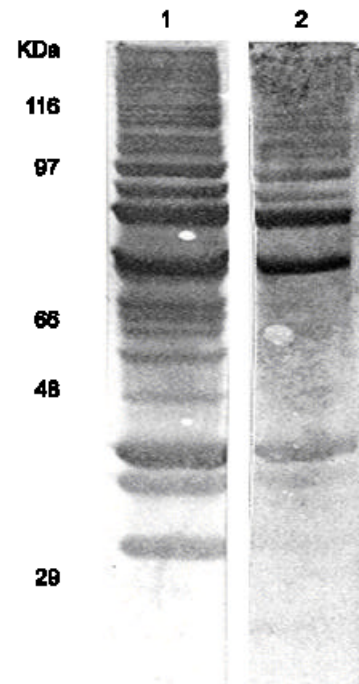


Fig. 2: Immunoblot analysis of *M. gallisepticum* S6 strain antigens with IgY. Lane 1 represents antigens treated with polyclonal anti-*M. gallisepticum* primary IgY from immunized chicken. Lane 2 represents antigens treated with egg-derived IgY of adjuvant-immunized chicken. Numbers to the left of the gel show the sizes of molecular mass markers.

The capacity of IgY to protect chickens against experimental *M. gallisepticum* infection was tested. Chickens infected with *M. gallisepticum* S6 strain were subsequently passively immunized with three different concentrations of anti - *M. gallisepticum* IgY daily for seven successive days. A dose-dependent response relationship was obtained where increasing IgY titer in passively immunized chickens decreased the isolation rate of the challenged strain S6. Compared with that in control chickens, bacterial isolation prevalence was

significantly ( $P < 0.05$ ) lower among chicken given IgY with titers of 15 µg/ml and 31 µg/ml. These findings support our hypothesis and confirm that IgY specific for whole cell *M. gallisepticum* F strain are partially protective against infection with heterologous *M. gallisepticum* S6 strain infection when given in sufficiently high concentration and thus, may be clinically useful as an immuno-therapeutic agent during Mycoplasmosis outbreaks.

In this study, IgY obtained from hens immunized with *M. gallisepticum* F strain whole-cell lysate, dramatically inhibited the growth of *M. gallisepticum* *in vivo*. It seems clear from our findings, using chicken, that IgY decreased morbidity. If the antibody IgY had no specific effect, no inhibition of bacterial growth would occur in immunized chicken. However, it is not clear how IgY protected the infected chickens against *M. gallisepticum* infection and the mechanisms by which IgY prevented *M. gallisepticum* colonization are yet not elucidated.

It is well known that the adhesion of *M. gallisepticum* to its host target cell is a crucial, initial step for the establishment of the disease. Therefore, since *M. gallisepticum* infection is initially a respiratory infection, the choice of intraperitoneal route for IgY inoculation might not be the best presentation. However, it is at some stage transiently systemic. Subsequent to the colonization of the respiratory tract, *M. gallisepticum* translocates across the respiratory mucosal barrier, enter the bloodstream and progress to systemic infection throughout diverse body sites of infected birds (Lamas da Silva and Adler, 1969; Bencina and Dorrer, 1984; Soeripto *et al.*, 1989; Chin *et al.*, 1991; Simecka *et al.*, 1992). Passive transfer of *M. gallisepticum*-specific IgY to the mucosal surfaces of birds following pathogen challenge might have thwarted the invasion of pathogens into the cells. Thus, by binding to a pathogen, systemically delivered antibodies inhibited pathogens from widespread colonization of host cells. Therefore, the therapeutic value of IgY against the experimental infection in chicken perhaps lies in its ability to inhibit the bacterial organism colonization. However, the relationship between inhibitions of bacterial activity and adhesion properties needs to be clarified.

In conclusion, the present study investigated the efficacy of IgY against *M. gallisepticum* infection in a controlled trial in chickens. However, possibilities of passive immunization via intraperitoneal injection under practical conditions in big chicken farms would have to be tested. If *M. gallisepticum*-specific IgY used as the sole agent in preventing or treating Mycoplasmosis in chickens, affected broiler breeder flocks should be immunized with IgY prior to onset of infection in order to increase the protection rate. Even though *M. gallisepticum* is one of the major health problems facing the poultry industry throughout the world, the results of this study indicate that IgY derived from eggs of hens immunized by *M.*

*gallisepticum* may provide an alternative approach to the management of *M. gallisepticum* infections in chickens. However, in order to reduce losses due to adverse affects of *M. gallisepticum*, IgY should be integrated in combination with other established means of prevention such as sound biosecurity programs.

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