

Antibody Pretreatment Does Not Impact *Mycoplasma Gallisepticum* Vaccination or Experimental Infection

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Abstract

Mycoplasma gallisepticum infection of poultry results in chronic respiratory disease in chickens and infectious sinusitis in turkeys. Infection leads to decreased egg production and increased carcass condemnation, resulting in decreased profits for producers. Vaccination against *M. gallisepticum* provides protection against the pathogenic effects of *M. gallisepticum* infection. However, many questions about the protective nature of the host immune response exist. This work addresses the role of antibodies during the initial host response to *M. gallisepticum* infection. F-strain *M. gallisepticum* vaccine and virulent R-low *M. gallisepticum* were exposed to growth-inhibiting antiserum for 30 minutes prior to vaccination or infection to determine their impact on vaccine efficacy and disease pathology. The results of this work show no significant differences due to serum pretreatment, suggesting that exposure to anti-*M. gallisepticum* antibodies during the initial stages of host colonization is insufficient to impact vaccination or infection outcomes.

Keywords: *Mycoplasma gallisepticum*; Vaccination; Antibody; Serum

Introduction

Mycoplasma gallisepticum (MG) is a significant pathogen of poultry and other avian species [1,2]. Infection with MG can cause chronic respiratory disease in chickens and infectious sinusitis in turkey, leading to reduced feed efficiency and egg production and increased carcass condemnation, resulting in increased costs to producers and consumers [3,4]. Control of MG is difficult. Antibiotics provide limited relief from MG infection, but complete elimination of MG from an infected flock is nearly impossible using antibiotics [3,5-7]. Live and killed vaccines are also commercially available. Killed vaccine efficacy is only of short duration with limited protection [8]. Live vaccines have been shown to be highly effective at preventing the most pathological effects of MG infection, with more efficacious vaccines causing limited pathologic effects compared to less efficacious vaccines [1,8].

The protective effects of vaccination against MG are not well understood [3]. Some work has pointed to the importance of the cellular and innate immune response to MG infection [9,10]. Results detailing the importance of the humoral immune response have been mixed [3]. Some studies show a poor correlation between circulating antibodies and protection [11-13]. Other results point to the importance of the local humoral immune response as well as the bursa of Fabricius in the host response to MG [10,14,15]. One study demonstrated that respiratory tract washing provides significant protection from MG in tracheal-organ-ring culture in a dose-dependent manner [16]. It was hypothesized that local antibody response inhibits MG attachment to the respiratory tract [16]. Therefore, the following study was devised to determine the effect of anti-MG antiserum locally applied to MG bacteria during the subsequent vaccination or infection of the chicken host.

Materials and Methods

Animal Care and Management

Hy-Line W-36 chicks (mixed sex) were obtained from a commercial MG and *Mycoplasma synoviae* free source. Chicks were placed on clean pine shavings in a conventional house and were raised according to Hy-Line W36 management guidelines [17]. Chicks were fed a standard layer diet regimen that met or exceeded nutritional requirements for that age as described previously [18,19]. The care and management of all chickens was performed using protocols approved by the USDA-Agricultural Research Service Institutional Animal Care and Use Committee (Mississippi State, MS location).

Experimental Design

At eight weeks of age, thirty mixed sex chickens were randomly divided between 6 isolation units [20], with 5 chickens per isolation unit and each unit with 5 chickens as a treatment group. Treatment groups include: Control (no treatment), Vaccine Control (vaccinated, no antiserum pretreatment), 1X Serum Vaccine Control (vaccine pretreated with antiserum prior to vaccination), Sham Vaccination + Challenge (sham vaccination including antiserum at 8 weeks of age plus MG challenge, no antiserum pretreatment), Challenge (MG challenged, no antiserum pretreatment), 1X Serum Challenge (MG challenge pretreated with antiserum).

Chickens were vaccinated with *M. gallisepticum* vaccine AviPro® MGF (*M. gallisepticum* F-strain based vaccine, Lohmann Animal Health Int., Winslow, Maine) at time of placement. All vaccine was administered as 20 µl doses to the right eye. Chickens were challenged with MG strain R-low at 14 weeks of age. The MG vaccine or challenge strains were pretreated by incubation with antiserum generated against *M. gallisepticum* F-strain for 30 minutes prior to vaccination or challenge, as indicated by the Vaccine or Challenge group label. At 15 weeks of age, all chickens were bled for serum, killed, and examined for airsacculitis. Serum plate agglutination (SPA) assay was used to quantitate the host humoral immune response to MG. Gross airsacculitis was scored on a scale from 0-3 as described previously [21,22].

Statistics

All data were log₁₀ transformed prior to statistical analysis. Both airsacculitis and SPA data were analyzed using the one way ANOVA function of SAS Enterprise Guide [23]. The significance of differences between the treatment groups means was separated using Tukey's studentized range test. The results were considered to be significantly different if P≤0.05.

Results

An initial experiment was performed in our laboratory to measure the growth inhibition of the anti-MG F-strain antiserum on MG strain R-low. It was determined that supplying the serum at 20% of the total growth media completely inhibited the growth of MG strain R-low (data not shown). This serum concentration is similar to that used in most growth medium for mycoplasma. The next lowest dilution at 2% did not inhibit MG growth (data not shown). For the live animal study, a 1-1 mixture of serum and MG culture were incubated together for 30 minutes prior to vaccination or challenge.

Results show that pretreatment with anti-MG antiserum did not impact the function of the vaccine. The SPA results were identical for both vaccinated groups (Figure 1A). SPA results were slightly different for the unvaccinated challenged groups. In this case, there did appear to be a slight but not statistically significant decrease in the SPA response when the challenge dose was pretreated with anti-MG serum. However, the SPA results for the sham vaccination + challenged chickens were expected to be identical to the challenge and 1X serum challenge chickens. Their results were lower although also not statistically different than the anti-MG serum treated challenge group, suggesting that the variation in results is simple experimental variation at an early time point post-MG exposure and not due to an actual effect of anti-MG serum treatment. The results for airsacculitis essentially mirrored the SPA result (Figure 1B). The presence of anti-MG serum showed no statistical difference in causing airsacculitis, or the ability of the vaccine to prevent airsacculitis.

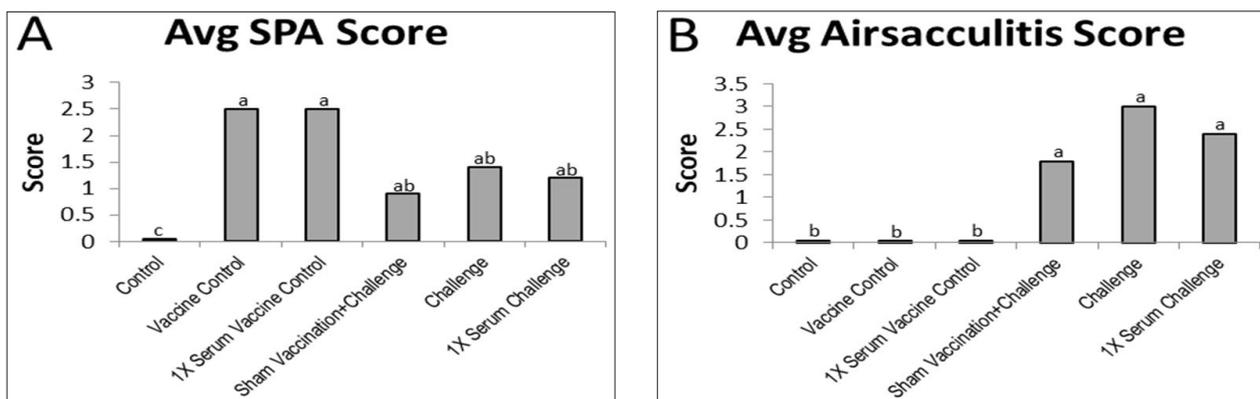


Figure 1: Serology and pathology results following *M. gallisepticum* vaccination and infection
 (A) Average SPA scores at 6 weeks post vaccination and 1-week post infection (15 weeks of age). SEM=0.2171. Bars with differing superscript letters are significantly different
 (B) Average airsacculitis scores at 6 weeks post vaccination and 1-week post infection (15 weeks of age). SEM =0.2729. Bars with differing superscript letters are significantly different

Discussion

Vaccination is an effective and efficient means of protecting poultry against MG infection however; the resulting specific protective effects of the host immune system have not been clearly identified. Some evidence points toward the involvement of the innate immune system for protection during the initial stages of infection. Bonneaud, *et al.* demonstrated a shift in expressions of genes influencing innate immune expression in naïve finches after many years of that finch population being

exposed to MG [24]. Naïve finches from a separate, unexposed population did not exhibit the same innate immune response, demonstrating a role for the innate immune response in MG disease resistance [24]. Other research demonstrates that protection by vaccination is effective in the absence of a significant systemic antibody response, suggesting a role for the cellular immune response [25]. This is supported by the work of Gaunson, *et al.* who suggest a role for both natural killer cells and cytotoxic T cells in response to MG infection [9,10].

Research on the role of the humoral immune response has yielded mixed results. Research has suggested that the systemic humoral immune response likely has only a minimal role in protection from MG infection. Work by Elsheikha, *et al.* demonstrated that the adoptive transfer of purified IgY antibodies to a naïve host resulted in decreased rates of MG detection [26]. The decrease in the rate of detection correlated with the dose of anti-MG antibodies, with larger doses of purified antibodies resulting in decreased rates of detection. This differs somewhat from work by Talkington and Kleven who showed that vaccination against MG decreased isolation rates of an MG challenge strain, but that there was no correlation between levels of antibodies in the host and the isolation rate of MG following challenge [12]. Work by Lin and Kleven demonstrated that passive immunization with anti-MG serum could decrease MG caused pathology, but only if very high doses of high-titered antiserum were used [11]. Lower amounts of antiserum or antibody had no significant effect on disease pathology. This appears to agree with work by Noormohammadi, *et al.* showing protection from MG induced pathology by vaccinations that resulted in minimal host system humoral immune response against MG [25]. This corresponds well with research from other mycoplasmas, including recent findings that passive immunization does not protect against experimental *Mycoplasma haemofilis* infection [27].

While the system humoral immune response may not be a major factor in protection from MG infection, there is evidence to suggest that the local humoral immune response may be involved in protection, at least during the initial stages of infection. Early work by Slavik, *et al.* showed that tracheal washings contained a substance that inhibited the cilia stopping effects of MG on tracheal organ culture [28]. Their work suggested that secretory IgA was not involved. A similar study by Avakian and Ley showed the involvement of MG-specific immunoglobulins in inhibiting MG from attaching to tracheal ring organ cultures in a dose-dependent manner [16]. Their work also suggested that IgA titers were unimportant. Works by Papazisi, *et al.* and Javed, *et al.* suggest that the local humoral immune response in the trachea resulted in significant of IgA and IgG produced in the trachea, which may be responsible for blocking colonization by MG during the initial phases of infection [29,30]. These results may explain those obtained by Elsheikha, *et al.* as their passive immunization protocol may have resulted insufficient levels of antibody in the respiratory organs to inhibit MG binding and therefore decrease MG detection levels [26]. Work by Yagihashi, *et al.* used an intratracheal vaccination component resulted in decreased MG isolation rates and decreased tracheal lesions scores compared to unvaccinated controls or a vaccination regimen that did not include an intratracheal component, further supporting the role of localized antibodies in host protection [31].

The pretreatment of the mycoplasma with antiserum and application to mucosal surfaces performed in this study should also have inhibited MG adherence to and colonization of the host respiratory mucosal surfaces as well. However, the results presented here suggest that while inhibition of binding and colonization may have occurred, it was insufficient to influence the effects of vaccination or infection. The transient nature of the antiserum exposure suggests that the role of localized antibodies during initial MG infection is to prevent adherence to the host rather than further interactions with the host immune system such as complement activation or antibody-mediated phagocytosis. This supports the observations of Javed, *et al.* [30].

Conclusion

The results of this work suggest that the presence of the antibody on the surface of the mycoplasmas is insufficient to prevent a host response to the vaccine or the development of MG induced pathology. However, a decrease in MG interacting with the host or other effects of anti-MG antiserum may be occurring that could only be detected with fractional vaccine doses, decreased MG R-low infectious doses, or a shorter time frame for measuring results.

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