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Maternal transfer of immunity: role in *Mycoplasma hyopneumoniae*

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Introduction

Enzootic Pneumonia is one of the most important contributors to disease-associated loss in swine production and the condition is present in almost every country were pigs are raised (Ross, 1999). *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) was initially established as the causative agent of Enzootic Pneumonia in pigs (Mare and Switzer, 1965) (Goodwin et al, 1965). Mycoplasmal pneumonia is characterized by early respiratory signs, usually seen in mid to late nursery and is seen mostly in herds with continuous flow and where multiple aged pigs are housed in the same site. However, the disease has changed in recent years, showing a more severe and later (finishing) presentation, which has been designated Porcine Respiratory and Disease Complex (PRDC). PRDC is seen in offsite weaning systems, where same-age groups are reared separate from other pigs (Pijoan, 2005). *M. hyopneumoniae*'s economic impact on the industry is not only related to the direct effects of the agent, but also complications with other respiratory pathogens, bacterial as well as viral. For these reasons, herd Mycoplasma status and disease intervention are important topics.

M. hyopneumoniae is transmitted by direct contact between pigs (respiratory tract secretions from infected swine) and all ages are equally susceptible (Rautiainen and Wallgren, 2001). *M. hyopneumoniae* infections can result from vertical as well as horizontal transmission (Ross et al 1999). Sow to pig transmission occurs during the lactation period. It has been hypothesized that sow to piglet infection is established in few pigs, and that later penmate contact disseminates the infection within the herd. In offsite systems, this internal dissemination is the only likely source of infection. Conversely, continuous flow systems can have considerable lateral infections from older pigs, resulting in dissemination of infection and early disease. Contact infection between penmates is slow. Recent data (Meyns, 2004) showed that the calculated R_n (transmission probability) for *M. hyopneumoniae* during the nursery period (6 weeks) was only 1.16. When infection of *M. hyopneumoniae* is confirmed in a herd, several strategies can be applied as attempts to manage the condition. Usually, there are two control approaches taken towards stopping or more realistically, diminishing the infection: vaccination and antibiotic prophylaxis and treatment.

A considerable amount of information regarding control strategies for *M. hyopneumoniae* has been published. The data on control strategies comes from commercial antibiotic or vaccine efficacy trials, field reports and selective experimental studies. Very few controlled studies investigating Mycoplasma disease intervention appear to have been performed under field conditions using commercial herds. Antibiotics have been widely used in controlling *M. hyopneumoniae* infections. Effective antibiotics control the infection and allow the pig time to develop immunity to the agent. Additionally, antibiotic use for *M. hyopneumoniae* prevention may have beneficial effects for many other bacteria. *M. hyopneumoniae* vaccines, commercially available for years, are killed whole-cell preparations. Vaccines are applied subcutaneous or intramuscularly and are licensed for use both in the reproductive herds as well as marketing pigs (nursery and finishing). However, the use of sow vaccination is sporadic at best (Martellli et al, 2006).

Current *M. hyopneumoniae* control strategies are usually applied in the growing period, resulting in the use of large amounts of antibiotics. Vaccination of growing pigs in the nursery period may result in the spread of PRRS virus which makes timing of this vaccine difficult. A better approach to controlling *M. hyopneumoniae* would be to vaccinate and treat animals in the sow farm, which would reduce antibiotic as well as labor cost and would not result in dissemination of PRRS virus. Our group has shown that both sow vaccination (Ruiz et al 2003) and sow antibiotic treatment during lactation resulted in significantly lower prevalence of Mycoplasma PCR positive piglets at weaning (Fano et al 2005). The repercussion of our previous research is that lowering the prevalence at weaning results in a lower prevalence and severity of lung lesions at slaughter.

A potential problem associated with sow vaccination may be maternal antibody interference following piglet vaccination. The influence of maternal antibodies on response to vaccination in piglets has not been definitively established, however, several studies have documented that sow vaccination followed by suckling pig vaccination results in many pigs not seroconverting to the vaccine, suggesting that there is interference. However, these studies have

only evaluated post-vaccine seroconversion and have not evaluated if these piglets are protected against challenge. Currently, no correlation between serum antibody level and protection from bacterial colonization or Mycoplasma disease exist (Thacker et al., 1998; Haesebrouck et al., 2004). Also, there are no studies that have evaluated transfer of maternal cellular immune components such as T lymphocytes specific for Mycoplasma, which could play a significant role in the protection against this organism.

Maternal immunity in the pig

The epitheliochorial nature of the porcine placenta inhibits transfer of antibodies and cells from the dam to the fetus during pregnancy. Therefore, the newborn has a naïve immune system at birth and is dependant on passive transfer of maternal immunity to survive antigen challenge within the first few weeks of life. Maternal immunity is transferred to the newborn via colostrum and milk. Passive immunity consists of many immunomodulatory and antimicrobial factors including hormones, enzymes, immunoglobins, and cells.

The newborn's immune system is suppressed at birth due its immaturity, an imbalance in Th1/Th2 type responses, endocrine factors, and due to passive interference by maternal antibodies. Passive interference can be due to direct effects of maternal antibodies binding antigens before neonatal immune system recognition or through indirect binding of receptors. Passive interference is a major cause for concern because it disallows the neonatal immune system from mounting a response as well as from preparing for future antigen challenge. An unrecognized, presumably important component of passive immunity are lymphoid cells delivered to the newborn via colostrum (Le Jan, 1996). There are more than $1x10^7$ cells per ml in colostrum and milk and it is estimated that piglets obtain 500-700 million maternal cells daily (Evans et al, 1982; Magnussen, 1999). Lymphoid cells are absorbed through the intestinal wall but are highly selective in their transport. Little is known about interference regarding passively transferred cells or the role these cells may play in immune development of the newborn (Bandrick and Molitor, 2005). The functional ability of colostral cells such as in vitro proliferation in response to mitogens as well as bacterial and viral antigens has been observed (Wagstrom et al., 2000). Animal studies focusing on the functional role of colostral lymphocytes are limited; therefore, the function and biological significance of these cells in the newborn is unknown. Recent reports in rodents suggest that maternal derived T lymphocytes do not interfere with immune development of the newborn and in fact may be useful in overcoming the suppressive effects of passive immunoglobin (Siegrist, 2001).

Maternal immunity to *M. hyopneumoniae*

Symptoms of *M. hyopneumoniae* infection are usually seen in mid to late nursery piglets even though the pathogen is often transmitted pre-weaning. Vaccination is a useful tool in controlling enzootic pneumonia; however, passive immune interference has many implications for vaccination of young pigs. While many studies have evaluated the value of humoral immunity in conferring protection from *M. hyopneumoniae*- associated disease, the role of cell-mediated immunity in protecting animals from enzootic pneumonia remains largely unknown.

Since *M. hyopneumoniae* attaches to the mucosal epithelium, a cell mediated immune response may be important in controlling and clearing infection. Studies have demonstrated increased *M. hyopneumoniae*-specific IFN-γ production (Thacker et al., 2000) and lymphocyte proliferation (Kristensen et al., 1981) by T-lymphocytes stemming from vaccinated pigs compared to non-vaccinated pigs, suggesting that T lymphocytes are important in immunity to *M. hyopneumoniae*. No studies, however, have evaluated the role of maternal *M. hyopneumoniae*-sensitized cells in protecting neonates from *M. hyopneumoniae* challenge. We hypothesize that *M. hyopneumoniae* prevalence at weaning can be lowered by applying different control strategies in the sow herd and/or suckling piglets. It is the goal of this study to evaluate the effect of a combination of control measures including vaccination against *M. hyopneumoniae* in a commercial setting, as well as to examine passive transfer of antibodies and immune cells against Mycoplasma from the sow to her offspring. A greater understanding of the role of maternal immunity in protection of neonates and potential interference with active immunity will provide insights into more effective use of control strategies.

Intervention strategies for *Mycoplasma hyopneumoniae*: role of maternal immunity

Ideal timing of vaccination must balance complications of interference associated with passively acquired maternal immunity and pathogen exposure. In most vaccination regimens timing is most often correlated with antibody titer without regard to cellular immunity. While vaccination is practiced to control *M. hyopneumoniae* infections, the protective immune response(s) have not been elucidated. Studies have demonstrated a possible contributive role of lymphocytes to the anti-Mycoplasma immune response but have not evaluated the ability of colostral cells to respond to Mycoplasma or to protect from infection in the newborn pig.

This study is being undertaken to assess vaccination timing and strategies (sows and/or piglets) as interventions to

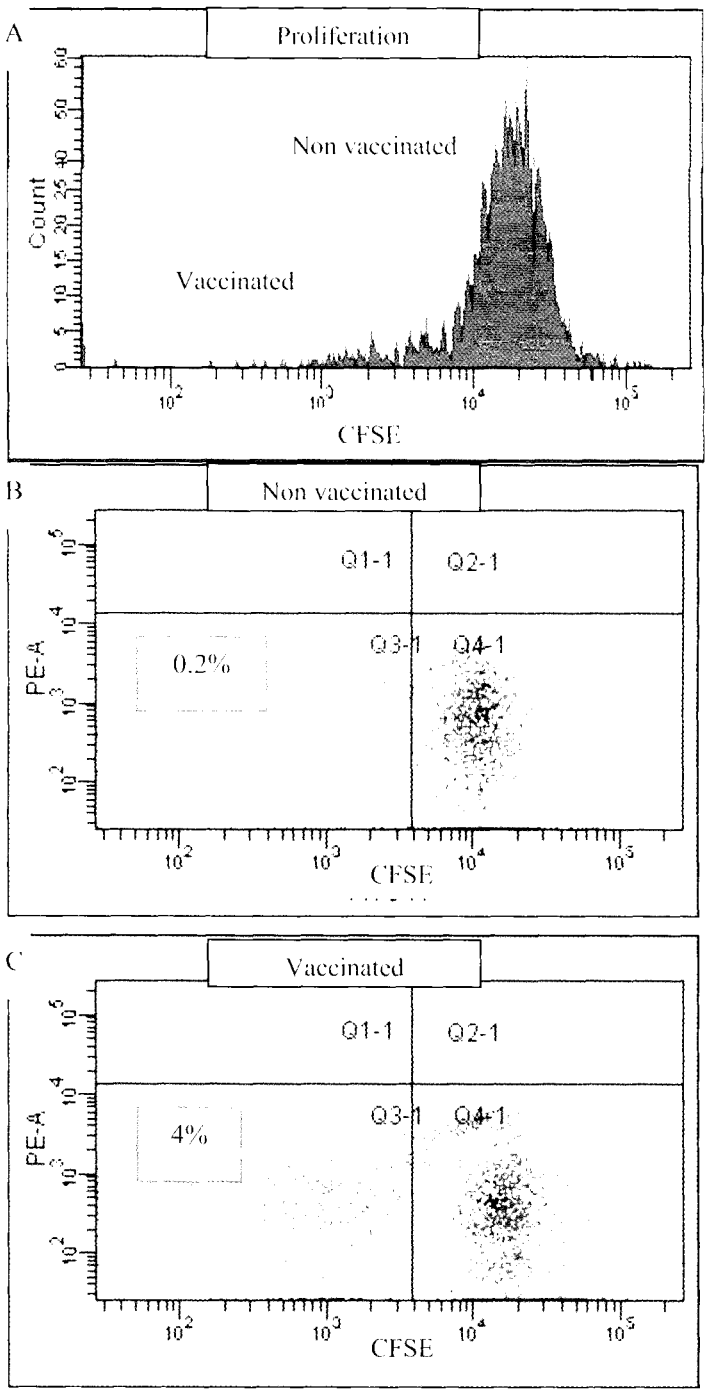
control *M. hyopneumoniae* infection and to determine if sow immunity exerts an effect on piglet response to vaccination. PBMCs are isolated from piglets before and post colostrum and mid nursery. Piglets are +/- vaccination and from +/- vaccinated sows. To assess cell mediated immunity in vivo purified inactivated *M. hyopneumoniae* antigen is administered intradermally to induce a delayed type hypersensitivity (DTH) reaction. By performing DTH tests, we will be able to investigate the functional ability of colostral cells to respond to *M. hyopneumoniae* in otherwise naïve animals. Positive DTH responses in 4 day old animals will be the result of responding maternal cells since the young animal will not have developed its own sensitized cells at this time.

Preliminary evidence suggests that lymphocytes are primed by *M. hyopneumoniae* vaccination and respond to further stimulation with *M. hyopneumoniae* by proliferation and IFN-γ production. **Figure 1** is a representation of in vitro antigen specific proliferation by PBMCs from *M. hyopneumoniae* vaccinated and non-vaccinated pigs stimulated with inactivated *M. hyopneumoniae* antigen. In addition, sensitized lymphocytes are able to respond to purified inactivated *M. hyopneumoniae* in vivo by producing a DTH lesion. These results suggest that a cellular response may be important in the immune response to *M. hyopneumoniae* and transferred colostral cells may be important in protecting piglets from disease.

Conclusion and future directions

Mycoplasmal pneumonia caused by *M. hyopneumoniae* is one of the most common and economically important diseases in swine production. All pigs are susceptible to infection and the bacteria are transmitted vertically and horizontally. While control of *M. hyopneumoniae* often involves growing pig vaccination and antibiotic use, more effective intervention strategies to control *M. hyopneumoniae* infection are necessary. The definitive protective immune response to *M. hyopneumoniae* remains unknown; however, most intervention regimes are based on antibody levels in growing pigs without regard for cellular

Figure 1: PBMC response to in vitro stimulation with Mycoplasma hyopneumoniae antigen. PBMCs isolated from pigs vaccinated or not against *M. hyopneumoniae* were stimulated with purified *M. hyopneumoniae* antigen. Proliferation is demarcated by a decrease in the membrane stain CFSE. Cells stained with CFSE loose color in a stepwise fashion as they proliferate. A. Non-vaccinated animals are represented by the filled in area; vaccinated animals are represented by the dark outline. B. Non-vaccinated animals. C. Vaccinated animals.



immunity. Additionally, the role of passively acquired maternal cellular immunity in *M. hyopneumoniae* infection remains unclear.

Our current study helps to clarify the role of lymphocytes in the immune response to *M. hyopneumoniae* infection. This study will also shed light on the functional ability of colostral cells to respond in vitro and in vivo in an antigen specific manner. A limitation of the current study is that while it addresses the functional ability of sensitized cells to respond to *M. hyopneumoniae* antigen it does not asses the role of lymphoid cells in protecting from challenge. A future study is a challenge experiment to assess the role of maternal derived T cells in protection and interference. Since colostral cells cross into the piglet's circulation when transferred from sow to her progeny only, cross fostering piglets prior to colostrum ingestion will only allow the transfer of antibodies, not cells, into the piglet's circulation. At service sows will be divided into treatment groups, vaccinated and non-vaccinated; piglets will be divided into cross-fostered/vaccinated, non cross-fostered/vaccinated, cross-fostered/ non-vaccinated, non-cross-fostered/non-vaccinated groups. Cross fostering will occur at parturition before suckling and piglet vaccination will occur at processing and weaning. Piglets will be challenged with a virulent strain of *M. hyopneumoniae*; response to challenge will show how *M. hyopneumoniae* primed colostral cells influences piglet immunity including possible interference with active immune induction following vaccination.

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