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Passive immunity to *Mycoplasma hyopneumoniae*: transfer and protective role

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is a pathogen that affects pigs of all ages and has a worldwide distribution. Disease caused by this agent is commonly presented as a chronic pneumonia, called Enzootic Pneumonia. *M. hyopneumoniae* is associated with a low mortality rate; however, it becomes endemic in infected farms. One of the most important aspects of *M. hyopneumoniae* is that infection predisposes to colonization of the respiratory tract with other pathogens, bacterial as well as viral. *M. hyopneumoniae* infection affects the normal function of the mucociliary apparatus and produces a marked inflammatory response in the respiratory tract.

Swine farms become infected with *M. hyopneumoniae* first by the acquisition and introduction of carrier animals; subsequently infected sows colonize their offspring. The offspring after being weaned and mixed with susceptible pigs, will amplify the infection in the herd. In segregated production systems the most important means of *M. hyopneumoniae* transmission is by sow-to-piglet contact during the lactation period. However, the close relationship between sows and piglets during the lactation period also assures the transfer of immunity from the mother to the baby pig via colostrum. It has been recently demonstrated that *Mycoplasma hyopneumoniae* specific cellular immunity can be transferred from the immunized sow to her offspring.

Cross-fostering is a common management practice in which newborn piglets are moved among sows during the first 24 hrs after birth in an attempt to reduce litter variation and to increase the survivability of newborn pigs. The application of cross-fostering assumes that the balance of weight after birth within a litter is more important than normal development and performance individual pigs can have when left with their own mother. Extensive cross-fostering is usually observed in swine farms. However, several studies have demonstrated that excessive cross-fostering can have a detrimental effect in birth weight, growth and reproductive performance of gilts and can be stressful for piglets and sows. Controlled cross-fostering can be effective in minimizing the impact of infectious agents, such as porcine reproductive and respiratory syndrome virus and has been proposed as a management intervention for disease control.

The various measures that can be employed to control *M. hyopneumoniae* infections have been reviewed, among which vaccination appears to be the most widely used. Vaccination against Enzootic Pneumonia is applied in about 40% of the US swine herds and the bacterin is used worldwide. Although it has been shown that acquired immunity after vaccination with commercial bacterins decreases the detrimental effect of *M. hyopneumoniae* pneumonia, it is still unknown how the immune system does this. The specific action of antibodies and/or cells involved in the immune response to *M. hyopneumoniae* is not clear.

It is a common practice to measure the success or failure of *M. hyopneumoniae* vaccination by measuring seroconversion. It has also been proposed that the immune factors acquired by the piglet from its mother could be detrimental since passive immunity could interfere with an active immune response following vaccination. Nevertheless, a detrimental effect of maternally derived immunity in *M. hyopneumoniae* infection has not been demonstrated and several studies support the idea that maternal passive interference does not affect protection against *M. hyopneumoniae*; however it lowers the antibody response of the piglet.

To our knowledge, there are no published studies that have looked at either the effect of cross-fostering on the immune status of the newborn pig or to evaluate the protective role of antibodies and cells passively transferred to piglets against challenge with *M. hyopneumoniae*. Therefore, we designed two studies in which we evaluated the effect of cross-fostering newborn pigs at different times after birth on the transfer of cellular and humoral immunity to *M. hyopneumoniae* and evaluated the protective role of antibodies, immune cells or both immune components in protection against an experimental challenge infection with *M. hyopneumoniae*.

Evaluating the effect of cross-fostering on the transfer of *M. hyopneumoniae* maternal immunity

Four pairs of vaccinated and unvaccinated gilts and 4 pairs of vaccinated and vaccinated gilts had their offspring cross-fostered within the pairs. Two piglets at a time were moved from their mother to the corresponding paired gilt, from which 2 piglets were taken and relocated

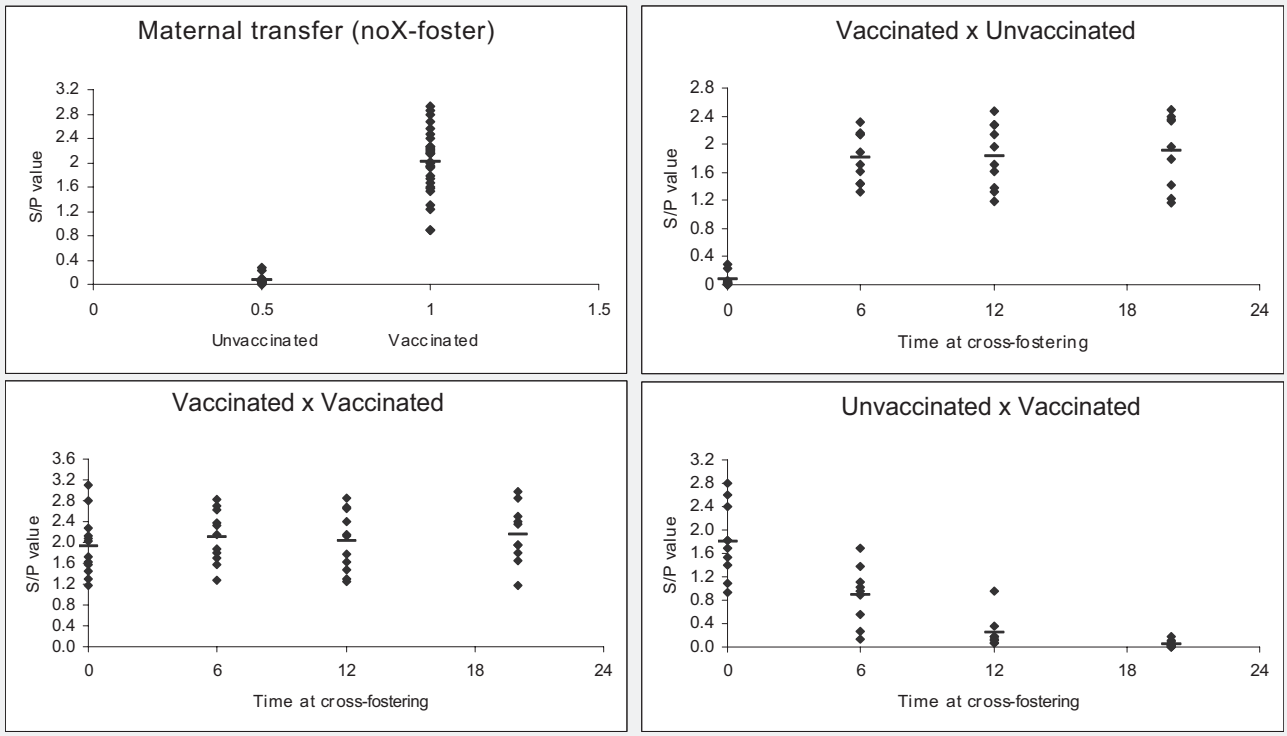
with the mother from whom the 2 piglets were originally taken. Piglets were cross-fostered at either 0, 6, 12 or 20 hrs after birth. Two piglets per litter were left with their genetic mothers. Piglets were subjected to detection of antibodies for *M. hyopneumoniae* by ELISA (HerdChek™ Mycoplasma hyopneumoniae Antibody Test Kit - IDEXX Laboratories, Westbrook, Maine, US) 24 hrs after birth. By 3–5 days of age all piglets were tested for *M. hyopneumoniae* specific delayed-type hypersensitivity (DTH).

The majority of piglets that received *M. hyopneumoniae* immunoglobins from the mothers absorbed detectable antibodies during the first 6 hrs after birth while only one piglet absorb those 12 hrs after birth (Figure 1). Piglets were able to absorb the antibodies no matter the maternal source (genetic or cross-fostered; Figure 1). A 24 hr period for absorbance of antibodies in piglets has been described in the literature. Our observations suggest that the antibody absorbance period in piglets may be different for antibodies generated against a specific pathogen or that the concentration of antibodies absorbed by the piglets 12 hrs after birth are undetectable by the most widely used ELISA *M. hyopneumoniae* antibody assay. On the other hand, cell mediated immunity resulted in more selective transfer, as previously shown. Collectively the results demonstrated that cross-fostering has a major impact on the transfer of immune components from the mother to her offspring. The justification for cross-fostering piglets is usually based on production parameters, e.g. to decrease pre-weaning mortality. Sparse information points out the importance of cross-fostering on disease dynamics and transfer of maternal immunity. Our study is the first investigation on the effect of cross-fostering on the transfer of pathogen specific immunity from the sow to the offspring. The criteria for piglet adoptions must combine aspects of production and immunity, as results from this investigation demonstrate a clear effect of allosuckling in regards to the absorption of *M. hyopneumoniae* specific immunity in the piglet.

Evaluating the protective role of maternal immunity against an experimental *M. hyopneumoniae* infection

Piglets carrying different immune components for *M. hyopneumoniae* can be found in field situations, for example, in a *M. hyopneumoniae* positive farm in which gilt vaccination and cross-fostering are practiced. A combination of gilt vaccination, piglet cross-fostering and colostrum treatment was employed to obtain groups of piglets with various immune statuses to *M. hyopneumoniae*: (1) Negative control. (2) Only immune cells. (3) Only antibodies. (4) Both cells and antibodies. (5) No immunity. Piglets in groups 2 to 5 were intra-tracheally infected with 1×10^5 ccu/mL of *M. hyopneumoniae*. Piglets were evaluated

Figure 1: *M. hyopneumoniae* antibodies in piglets detected by ELISA 24 hrs after birth. Results from treatment groups are represented in different panels. Samples with a sample to positive ratio (S/P) higher than 0.4 were considered positive, while samples with a S/P value lower than 0.4 were considered negative.



daily for coughing. Nasal swabs were collected from 5 piglets per group at several time points and 25 dpi all animals were euthanized, bronchial swabs were collected, lung lesions were scored. Nasal and bronchial swabs were processed for nested-PCR and real-time PCR.

The effect of antibodies and cells was evaluated alone and in combination. The artificial nature of the study made it difficult to obtain a sample size other than single litter treatment groups, therefore, only numerical differences where achieved when comparing clinical signs, bacterial shedding and bacterial load among groups. The trend was for piglets harboring *M. hyopneumoniae* specific antibodies and cells to show a slower and lower bacterial shedding than animals with no mycoplasmal immunity (Table 1). This trend is supported by the finding that animals harboring one or both immune components were not able to transmit *M. hyopneumoniae* to their mothers, while piglets in the no immunity group were able to transmit *M. hyopneumoniae.* A different scenario was observed when comparing lung lesions suggestive of *M. hyopneumoniae* infection. Piglets that received *M. hyopneumoniae* antibodies and cells showed the highest lung lesion score, which was significantly different than the score in all other infected groups. Lung lesions were not correlated with bacterial shedding and bacterial load in piglets, supporting the hypothesis that *M. hyopneumoniae* lung lesions are the result of an immune mediated reaction, also suggesting that bacterial replication is not the main factor responsible for the inflammatory process during the early stages of infection.

Conclusions

Under the conditions of our studies, *M. hyopneumoniae* specific antibodies are transferred from the mother to the piglet from 0 to 12 hrs after birth, regardless of the source (genetic mother or foster mother). Immune cells (*M. hyopneumoniae* specific) can be transferred from genetic mothers to piglets that remain with the mother during at least 12 h after birth. The onset of coughing and coughing score were similar in *M. hyopneumoniae* infected piglets in spite of the various mycoplasma specific immune statuses of the piglets. Lung lesions associated with *M. hyopneumoniae* infection were more severe in piglets carrying mycoplasmal immunity than in piglets with no immunity or only cells or antibodies. Piglets harboring *M. hyopneumoniae* specific antibodies and cells showed a numerically slower and lower bacterial shedding than animals with no mycoplasmal immunity.

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Table 1: Detection of *M. hyopneumoniae* DNA in nasal and bronchial swabs, from 0 to 25 dpi.

	Nasal swabs						
Treat. group / dpi	**0**	**7**	**10**	**14**	**21**	**25**	**Bronchial swab (25 dpi)**
No cells + No Ig's	0/5	0/5	2/5	2/5	2/5	3/5	8/9
Only cells	0/5	0/5	2/5	4/5	4/5	4/5	7/8
Only Ig's	0/5	0/5	1/5	1/5	2/5	3/5	9/9
Cells + Ig's	0/5	0/5	0/5	1/5	1/5	3/5	8/9

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