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Layout
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Logo Design
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Cover Design
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Experiences with *Haemophilus parasuis*

Simone Oliveira and Carlos Pijoan

Department of Clinical and Population Sciences, College of Veterinary Medicine, University of Minnesota

Introduction

Haemophilus parasuis is an early colonizer of the upper respiratory tract of swine (1). This organism can be normally isolated from the nasal cavity, tonsillar area and trachea of healthy animals (2, 3). Control of *H. parasuis* infections can be attempted by preventive vaccination of susceptible animals. However, there is no satisfactory vaccine against *H. parasuis* so far. The lack of cross-protection between *H. parasuis* strains and serovars and the large number of non-typable strains makes the development of a universal vaccine a difficult task. Moreover, constant introduction of replacement animals into a naïve population can bring new pathogenic strains into the herd, which may not be included in the vaccine. In order to clarify some important points regarding the development of protective immunity against *H. parasuis*, this report will discuss the epidemiology of the agent within a herd, the role of colonization in disease control, the role of commercial and autogenous vaccines in protection and potential virulence factors associated with systemic infections.

Epidemiology of *H. parasuis* infections within a herd

The epidemiology of *H. parasuis* infections can vary depending on the sanitary status of the herd and management strategies adopted. In conventional herds, where the prevalence of colonized animals and the diversity of *H. parasuis* strains are high, systemic infections are sporadic, stress-associated, and affect mainly young animals (4). In specific pathogen-free (SPF) or herds with high-health status, the epidemiology of *H. parasuis* infections changes considerably. The prevalence of colonized animals and the variety of *H. parasuis* strains is limited, which results in lack of protection against new strains.

Genotyping studies have shown that in a multi-farm outbreak the number of pathogenic strains of *H. parasuis* isolated from systemic sites of diseased animals is very restricted (5). In our experience, we have noticed that the epidemiology of *H. parasuis* infections is a dynamic process, and that the pathogenic strains causing disease in a population can change over time. Another interesting aspect of the epidemiology of *H. parasuis* infections is that although animals are colonized with a variety of serotypes and strains, the herd's pathogenic strains have a low prevalence in the sow population (2). Consequently, a small number of piglets are colonized with these strains prior to weaning. This particular feature of the epidemiology of *H. parasuis* explains in part why systemic infections generally occur after weaning, when colonized animals are mixed with non-colonized animals.

Use of colonization with *H. parasuis* for disease control

Natural exposure to *H. parasuis* is the best way to confer full protection against systemic infections. A recent study has demonstrated that colonization by *H. parasuis* occurs as early as a few hours after birth and that intensity of colonization and number of colonized animals increase over time (Oliveira 2001, unpublished data). As discussed in the previous section, the prevalence of the pathogenic strains of *H. parasuis* in the sow population is very low. The use of SEW reduces the chances of natural colonization of piglets by sows and increases the number of naïve animals entering the nursery. Disease caused by *Haemophilus parasuis* often occurs after mixing of animals from different origins or, in the case of weaning, after mixing piglets from different sows with different levels of colonization.

In order to better define if early colonization of piglets with the herd's pathogenic strains of *H. parasuis* reduces disease in the nursery, we have conducted a study (2) in which two protocols of colonization were tested:

- direct colonization of piglets, and
- colonization of piglets through nose-to-nose contact with inoculated sows.

Haemophilus parasuis isolates recovered from diseased nursery pigs were characterized by the rep-PCR technique and the herd's prevalent strain was used for colonization. Piglets in the experimentally colonized groups were inoculated with a 7(10^3 CFU/ml dose at 5 days of age by the oral route using a spray pump. Sows were colonized at 2 weeks prior to farrowing using a similar protocol. Although both colonization protocols were successful in getting the piglets colonized, direct inoculation of five-

day-old piglets with the herd's pathogenic strain of *H. parasuis* tended to be more effective in reducing the morbidity and the mortality than the colonization of piglets by nose-to-nose contact with inoculated sows. This study demonstrated the importance of early colonization of piglets in the development of protective immunity against *H. parasuis* in the nursery period. However, in field conditions colonization of young animals is resultant of the exposure of piglets to colonized sows, which based on our results, is less effective then direct colonization of piglets to confer protection in the nursery.

Vaccination and protective immunity against *H. parasuis*

Considering that colonization of piglets prior to weaning is partially effective in field conditions, vaccination against *H. parasuis* becomes an important measure of control of systemic infections in the nursery. Commercial and autogenous vaccines can be used to control *H. parasuis* infections. Vaccination against *H. parasuis* is very successful when the vaccine strain is homologous to the herd's pathogenic strain (6, 7). If the *H. parasuis* serotypes that are causing systemic disease in the herd are included in the selected commercial vaccine, chances are that vaccination will be protective. However, use of commercial vaccines is not always successful. Lack of effectiveness of commercial vaccines may be related with several factors. The introduction of new serotypes or strains into the herd and the presence of non-typable isolates are some of the factors that may influence success of vaccination. Other important aspect is the heterogeneity of *H. parasuis* strains even within of the same serovars group (8, 9) When commercial vaccines are not effective, use of autogenous vaccination can be helpful for disease control. The use of an autogenous vaccine requires an epidemiological evaluation of the herd in order to determine the prevalent strains causing disease. For an accurate characterization of the pathogenic strains present in the herd serotyping is a limited technique, especially if non-typable strains are involved in disease. Considering that *H. parasuis* can be normally isolated from the upper-respiratory tract of healthy animals, it is imperative that only strains recovered from systemic sites like brain, pericardium, pleura, peritoneum and joints be submitted for epidemiological evaluation. The rep-PCR technique has been used with success for epidemiological studies in herds affected by *H. parasuis* infections (10). This genotyping technique allows full characterization and comparison of systemic *H. parasuis* strains and can be used to identify the prevalent strains present in the herd. By using the rep-PCR technique, the introduction of new strain into the herd can be monitored by routine evaluation of new isolates recovered from diseased animals. New prevalent strains can then be added to the autogenous vaccine.

As was discussed before, the success of commercial or autogenous vaccines for control of *H. parasuis* infections will depend on the epidemiology of the agent within the herd. Some herds have experienced failure in vaccination after adopting both vaccines. In these cases, the presence of concurrent diseases, like PRRS for example, has to be investigated. If PRRS infection is active in the herd, chances are that both commercial of autogenous vaccines will failure until PRRS is controlled.

Virulence factors in *H. parasuis* infections and importance in development of protective immunity

The ideal vaccine against *H. parasuis* would promote full protection against homologous and heterologous challenge. Several vaccine trials reported in the literature demonstrate that vaccination against *H. parasuis* induces satisfactory protection against homologous challenge, but not against heterologous infections (6, 7). Cross-protection between *H. pàrasuis* strains and serovars is restrict and frequently unsuccessful. Two major factors are involved in lack of cross- protection between different *H. parasuis* strains or serovars:

- It is known that some *H. parasuis* strains or serovars are considerably more virulent than others. However, virulence factors expressed by *H. parasuis* during infection are largely unknown.
- The diversity of serovars and strains of *Haemophilus parasuis* makes the development of a unique vaccine a difficult task.

Some virulence factors present in other organisms from the *Haemophilus-Actinobacillus-Pasteurella* (HAP) group have been described for *Haemophilus parasuis*. However, the role of these factors in infection has not been established. Munch *et al* (1992) describes the expression of fimbriae by *H. parasuis* strain Bakos A (serotype 2) after *in vivo* passage in 10-day-old embryonated eggs (11). Other important virulence factor expressed by organisms included in the HAP group is the expression of capsule during infection. Capsulated strains are known to be more resistant to phagocytes and complement fixation than non-capsulated strains (12). The association between capsule expression by *H. parasuis* and virulence is very controversial in the literature. Morozumi and Nicolet (1986) report that most *H. parasuis* strains isolated from disease pigs were uncapsulated (13). Conversely, Rapp-Gabrielson et al (1992) noticed that a *H. parasuis* strain serotype 5 considerably increased capsule expression after *in vivo* passage in guinea pigs (14). The main difference between these two studies is that the first evaluated capsule expression after *in vitro* passages, while the second evaluated the presence of capsule after infection and an *in vivo* passage.

In order to further clarify if capsule expression by *H. parasuis* is triggered during infection, we have evaluated the presence of capsule in *H. parasuis* strains after *in vivo* passages using both the embryonated-egg and the pig models. In the first trial, a highly virulent *H. parasuis* strain serotype 5 was inoculated into a 10-day-old embryonated-egg. After incubation, *H. parasuis* was isolated in pure culture from the chorio-allantoidal membrane of inoculated egg. Acriflavine agglutination test and transmission-electron-microscopy (TEM) results showed that this *H. parasuis* strain considerably increased capsule expression after *in vivo* passage. The same strain was later inoculated into a colostrum-deprived piglet by the intraperitoneal route. The inoculated animal was necropsied 24 hours after infection and a sample of the peritoneal fluid was directly evaluated by TEM. Results showed that capsule expression during infection of the natural host was further enhanced compared with the egg-passed strain. This first trial showed that a highly virulent *H. parasuis* strain considerably increased capsule expression after *in vivo* passages and infection. To further evaluate capsule expression by other serotypes of *H. parasuis*, 7 *H. parasuis* strains were inoculated into embryonated eggs and evaluated by the acriflavine agglutination test. Results are summarized in **Table 1**. Results showed that two of the highly virulent strains (serotypes 5 and 14) recovered capsulation after *in vivo* passage. However, *H. parasuis* strains 1 and 10, previously reported as being virulent, did not express capsule after *in vivo* passage in embryonated eggs. Results obtained from these trials also demonstrated that virulent strains of *H. parasuis* enhanced capsule expression after *in vivo* passage. However, the role of capsule expression in *H. parasuis* virulence still needs to be clarified.

Some studies (13, 15) have suggested that virulent *Haemophilus parasuis* strains express similar outer membrane proteins (OMP), and that OMP patterns may be associated with virulence. Although it is clear that virulent strains are more homogeneous regarding OMP patterns than non-virulent strains (15), both groups of strains can express similar OMP patterns (**Figure 1**). Based on these observations, we can conclude that the role of OMP in *H. parasuis* virulence and induction of protective immunity still needs to be elucidated.

Final considerations

The development of a satisfactory vaccine against *H. parasuis* depends on knowledge of the virulence factors expressed by the agent. The main candidates for "virulence" are fimbriae and capsule expression and OMP patterns. However, none of these factors have been consistently associated with pathogenicity of *H. parasuis* strains. Natural exposure and colonization still are the best way to control *H. parasuis* infections in a herd, though commercial and autogenous vaccines can be used for disease prevention and control. Selection of the ideal vaccine will vary between different herds and will depend on the epidemiology of the agent within the target population.

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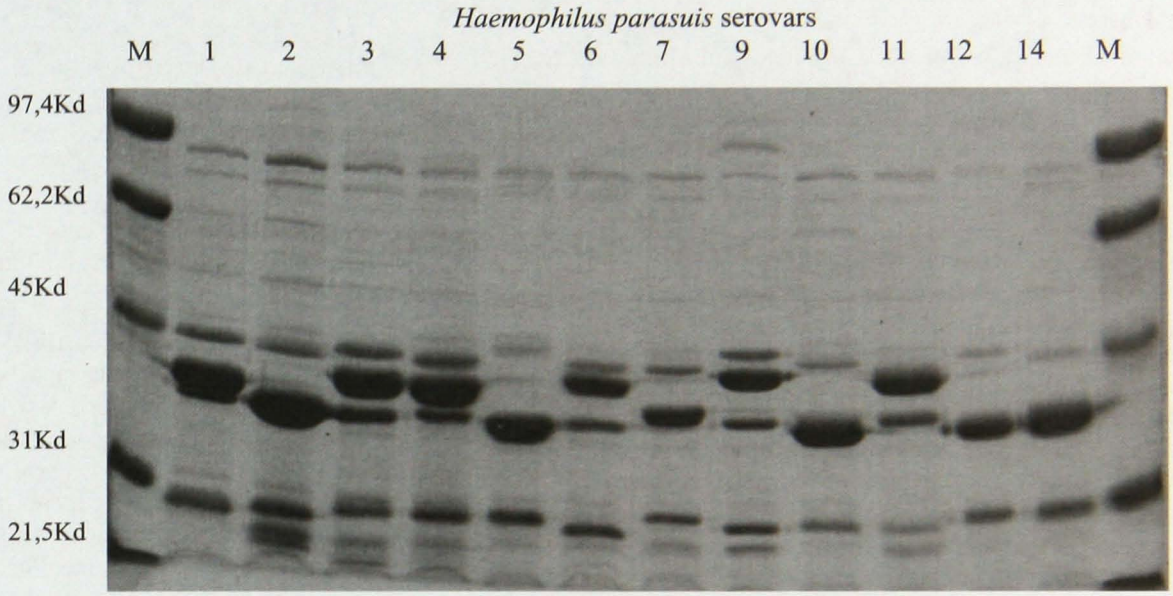
Table 1. Capsule expression of *Haemophilus parasuis* reference strains (serotypes 1, 4, 5, 7, 9, 10 and 14) before and after *in vivo* passage in embryonated eggs. Acriflavine agglutination test.

H. parasuis* strains*	**Reported virulence (4)***	**Presence of capsule before egg passage**	**Presence of capsule after egg passage**
Serotype 1	+ +	No capsule	No capsule
Serotype 4	+	No capsule*	No capsule
Serotype 5	+ +	No capsule	Capsulated
Serotype 7	-	No capsule	No capsule
Serotype 9	-	No capsule	No capsule
Serotype 10	+ +	No capsule	No capsule
Serotype 14	+ +	No capsule	Capsulated

* Intra-peritoneal inoculation.
(-) No clinical signs or clinical lesions.
(+) Clinical signs and lesions of polyserositis at necropsy.
(++) Death in 96h after infection.

Figure 1. Outer membrane proteins patterns of reference strains of *Haemophilus parasuis* representing different serovars



* Intra-peritoneal inoculation.
(-) No clinical signs or clinical lesions.
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