

Effect of pig age and autogenous sow vaccination on nasal mucosal colonization of pigs by *Haemophilus parasuis*

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Summary

This report describes an attempt to evaluate the effect of piglet age (with or without weaning), sow parity, and sow vaccination, on the timing of nasal mucosal colonisation of piglets by *Haemophilus parasuis*. We also determined the serotypes involved and compared serotypes isolated from the nasal mucosa and the trachea. Compared to tracheal swabs, nasal mucosal swabs were more easily performed and also more consistently yielded *H parasuis*. Serotypes obtained from each site were the same. A lower level of colonisation was found within the litters of young sows and a low level of colonization by *H parasuis* at weaning likely predisposes piglets to clinical disease in the nursery, assuming the presence of a virulent serotype. We found that more than one serotype of *H parasuis* was present in the herd and that vaccination of sows did not influence the timing of apparent colonization or the serotypes prevalent within a litter.

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Bacteria that colonize mucosal surfaces during the first two weeks of life have been termed early colonizers.¹ Assuming adequate transfer of specific colostral immunoglobulins, immunity against early colonizers (e.g., *Haemophilus parasuis*) is good in early lactation but declines progressively. Between about 10 and 15 days of age, antibody levels no longer prevent colonization, but do limit it such that clinical disease is prevented.¹ With

time, level of *H parasuis* colonization increases to the point where the pigs develop their own active immunity (about 3 weeks of age). Therefore, there is a balance between infection and resistance such that the piglets are subjected to a relatively slow and controlled exposure to a potential pathogen that allows them the time to develop their immunological defences.

Short lactations, high health status, and the isolated swine populations associated with modern swine production have increased the incidence and severity of disease due to infection with *H parasuis* (Glasser's disease).^{2,3} To be able to implement strategies to control *H parasuis* requires a knowledge of when pigs become colonized, the effect of piglet age on the proportion of the litter that becomes colonized, and the role of the immune status of the sow. An earlier study has demonstrated that, when the sows are free of *H parasuis* and hence fail to provide passive immunity to their litters, epizootic Glasser's disease may occur.⁴ More recent studies have shown that maternally derived passive immunity is essential for protection against *H parasuis* challenge³ but that sow immunity (and hence pig immunity via colostral immunoglobulins) following vaccination may be serovar specific.² While vaccination of sows may improve the level of protection afforded to the litter, it is possible that it may also prolong the period of slow colonization. For early weaned pigs, the effect of this could be a reduction in the proportion of a litter that is colonized at weaning and consequently an increased risk of clinical disease in the nursery. To further examine the relationship between sow immune status and piglet age, studies

were undertaken using a herd positive for *H parasuis*.

Case description

All pigs used in these studies were from the University of Alberta swine research unit and the experimental procedures were approved by the University of Alberta Animal Care committee. Herd composition consisted of a 100-sow commercial farrow-to-finish herd housed in a single barn, and a second component for reproduction research where sows were housed in separate breeding-gestation and farrowing barns and were killed prior to production of a second litter. Nursery rooms were operated all in-all out, by room. All buildings were on the same site. Gilts for both herd components were purchased from the same source and the lactation length was 25 to 28 days, except where indicated otherwise for specific experiments. Piglets from both components were co-mingled at weaning.

To confirm that *H parasuis* was present in the herd, nasal swabs were obtained from 11 randomly selected pigs in the nursery after wiping the nasal planum rostrale with alcohol. Swabs were submitted to the Alberta Agriculture bacteriology laboratory (Edmonton, Alberta) for culture. Nasal swabs were inoculated into modified Amies clear transport medium and delivered to the laboratory within 60 min of collection. Swabs were cultured to both enrichment (modified *Haemophilus* Mueller Hinton medium, H, Difco, Detroit, Michigan; [H]) and selective enrichment (as above with either 3 µg per mL vancomycin [HV] or 0.1 µg per mL crystal violet and 4.5 µg per mL bacitracin [HCB]) primary plating media in order to optimize recovery of *H parasuis*. Organisms were presumptively identified as *H parasuis* if they were V-factor dependent, positive for catalase, mannose, and saccharose, and negative for indole, urease, lactose, mannitol, raffinose, sorbitol, and CAMP; and if they grew luxuriously on H, HV, and HCB plating media.

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Haemophilus parasuis was isolated from 11 of 11 swabs cultured. Some colonies cultured from four of 11 swabs were identified as most likely to be *H parasuis*, since they showed the appropriate biochemical characteristics except that acid production in mannose and (or) saccharose was usually absent and, when present, was observed as a delayed weak reaction. Also, these colonies either failed to grow or grew sparsely on HCB medium.

Study One

The litters from eight sows were used. Four of the litters were derived from multiparous sows housed in the commercial production barn and were weaned at 28 days of age. The remaining four litters were derived from primiparous sows housed in the reproduction research farrowing barn and were weaned at 14 days of age. Within each weaning group, the nasal passages of all piglets from each of the four litters were swabbed at 14 and 28 days of age, and the swabs were cultured for *H parasuis*. Serotyping of isolates was not done.

All piglets were weaned into the same nursery, but each weaning age occupied the nursery at a different time. Pig health was monitored daily during the nursery period. At 14 days of age, 53% of piglets from multiparous sows were sufficiently colonized by *H parasuis* to yield a positive culture, while none of the piglets from primiparous sows yielded a positive culture (Chi square, $P < .001$). At 28 days of age, nearly all (97%) of the piglets that remained with their dams yielded a positive culture, but only 50% of pigs weaned at 14 days of age yielded a positive culture at 28 days of age (Chi square, $P < .01$). These results indicate that the level of apparent colonization was reduced either by low parity or barn and, further, that from 14 days of age, the rate of apparent colonization of pigs was slower if the pigs were weaned. Weaning delayed apparent colonization in this study, likely as a consequence of there being relatively few pigs in the nursery with a sufficient *H parasuis* load to allow efficient pig-to-pig infection. In contrast, unweaned pigs continued to have intimate contact with their dams, the presumed source of *H parasuis*. Eighteen days after weaning, a disease outbreak occurred in the pigs that had been weaned at 14 days, and three died. Post mortems revealed polyserositis, but a definitive etiology was not established.

Because the above data was confounded by parity and barn, we performed another replicate using four litters from each barn, weaned at 28 days of age, but all from primiparous sows. Piglets ($n=6$ or 7 per litter) were nasal swabbed at 14 and 28 days of age and the swabs were cultured for *H parasuis*. Serotyping of the isolates was not done. At 14 days of age, 39% of piglets in the commercial barn, and 25% of piglets in the reproduction research barn, were culture-positive for *H parasuis*, and this difference was not significant (Chi square, $P = .27$). By the time of weaning at 28 days of age, 96 to 100% of all piglets were culture-positive. The reason that the level of apparent colonization in the reproduction research barn was higher in this replicate than in the first was not resolved.

Study Two

This study was undertaken to determine the prevalent serotypes of *H parasuis* present within the herd and to examine the possibility that the serotypes identified would be different depending on whether the nasal cavity or trachea was swabbed. At 25 days (preweaning) and 50 days of age, 31 pigs were anesthetized with xylazine (2 mg per kg, IM) and ketamine (10 mg per kg, IM), and nasal cavity and tracheal swabs were collected. The tracheal swabs were guarded. If colonies were identified as either *H parasuis* or *H parasuis*-like, a representative colony of each bacterial type from each pig was submitted for serotyping (Bayer, Worthington, Minnesota). Additionally, colonies that appeared atypical on growth media and (or) biochemical reactions were also sent for serotyping.

Of the 31 nasal swabs obtained at 25 days of age, serotype 9 was isolated from 19 swabs, a non-typable serotype (NT) from one swab, and serotype 9 plus NT from 11 swabs. Serotype 9 was isolated from 14 tracheal swabs and NT from one tracheal swab. Sixteen swabs were negative for *H parasuis*. Only 29 pigs were swabbed at 50 days of age. Of the 29 nasal swabs obtained at 50 days of age, serotype 9 was isolated from 12 swabs, NT serotype from seven swabs, and both serotypes 9 and NT from eight swabs. Two swabs were negative for *H parasuis*. Tracheal swabs obtained at 50 days of age yielded seven serotype 9, one NT, and two serotype 9 plus NT. Nineteen swabs were negative for *H parasuis*.

When the organisms identified as most likely *H parasuis* were serotyped, all were *H parasuis* serotype 9. With the exception of two pigs at 50 days of age, tracheal swab isolates were always represented by the same serotype as that isolated from nasal swabs (e.g., if the nasal swab yielded either serotype 9 or NT or both, either serotype 9 and NT or both were isolated from the tracheal swab). However, the success of isolation from tracheal swabs was considerably less than that from nasal swabs. A comparison of *H parasuis* serotypes recovered from pigs swabbed at the two different ages revealed that six pigs carrying only serotype 9 at 25 days yielded only NT at 50 days. A further eight pigs that carried only serotype 9 at 25 days yielded both serotypes 9 and NT at 50 days. In contrast, ten pigs that carried both serotypes 9 and NT at 25 days yielded only serotype 9 at 50 days.

Study Three

The objective of this study was to determine the effect of maternal vaccination against *H parasuis* on the timing and cumulative incidence of apparent colonization of pig nasal mucosa by *H parasuis*. Of 17 sows housed in the commercial barn, nine received an autogenous anti-*H parasuis* vaccine at 5 and 2 weeks pre-farrowing, and eight served as unvaccinated controls. The vaccine was prepared commercially (Bayer Laboratories, Worthington, Minnesota) using one serotype 9 and one NT organism isolated during Study Two. The reason for using only one NT organism was that five NTs were determined to have the same outer membrane protein profiles, and so it was reasonable to conclude that they represented a single serotype (VJ Rapp-Gabrielson, personal communication). All pigs in each litter were nasally swabbed at 14, 21, and 28 days of age and the swabs were cultured for *H parasuis*. Representative colonies from two pigs from each litter were submitted for serotyping.

To confirm a greater intake of colostrum anti-*H parasuis* antibodies by pigs nursing vaccinated sows, each piglet was bled at 7 days of age and serum titers against *H parasuis* were determined by ELISA (Biovet, St. Anthony, Minnesota). Since the sow was the experimental unit, the mean titer for each litter was calculated and the treatment titer is the mean of the litter

Table 1: Effect of sow vaccination on proportion of litters having at least 50% of pigs colonized by *H parasuis* (HPS), on the serotypes recovered from the piglets, and on the anti-HPS titers in the piglets at 7 days of age.

	Number of litters colonized			Serotypes (No. of litters)	Anti-HPS titer * (\pm SE) in pigs
	14 days	21 days	28 days		
Vaccinated sows	5/9	9/9	9/9	9 (1), NT (4), 9 and NT (4)	10.1 \pm 2.9
Control sows	1/8	7/8	8/8	9 (1), NT (4), 9 and NT (3)	2.0 \pm 1.6

* Reciprocal of ELISA titer. Data represent the means of the average titer for each litter within treatment.

titers. Vaccination of the sow against *H parasuis* was associated with elevated anti-*H parasuis* titers in pigs 7 days of age (Table 1). There appeared to be no association between pig serum titers within a litter and the proportion of the litter that was apparently colonized at 14 days. Overall, there was no apparent effect of sow vaccination on the number of pigs within a litter that were apparently colonized by *H parasuis* at any of the ages examined. Similarly, there was no effect of sow vaccination on the serotypes of *H parasuis* recovered from the litters (Table 1).

Discussion

The present results serve to remind us that pigs nursing young sows will likely have a reduced exposure to early-colonizing normal mucosal flora. The potential clinical significance of this is illustrated by the example of a new herd that was farrowing too many primiparous sows for the available facilities. This necessitated a temporary shift in weaning age from 16 to 18 days to 11 to 14 days and, although a causal relationship was not proven, the nursery soon suffered an outbreak of Glasser's disease. Further, it is interesting to note that during the first replicate of Study One, an outbreak of disease occurred 18 days into the nursery phase, involving pigs weaned at 14 days of age. Three pigs died and post mortems were suggestive of Glasser's disease (polyserositis), although a *Streptococcus suis* etiology could not be ruled out. The occurrence of disease specifically in that group of pigs is consistent with the theory proposed by Pijoan,¹ that a low level of colonization by *H parasuis* (or *S suis*) at weaning predisposes piglets to clinical disease in the nursery.

The conclusions to be drawn from Study Two are that nasal swabbing provides an effective means of establishing the presence of *H parasuis* and the prevalent serotypes. Also, as previously shown by others,^{5,6} a herd may be infected concurrently with different serotypes of *H parasuis*, which may occur singly or in combination within a pig. Further, within the limits of the serotypes within a herd, the prevalent serotype within a pig may change over time.

Finally, the results of Study Three indicate that vaccination of the sow alone did not change the timing of apparent colonization by *H parasuis*, or the prevalent serotype. This implies that full protection at an early age requires vaccination of both the sow and litter as demonstrated by Solano-Aguilar et al.³

Implications

- Compared to tracheal swabs, nasal mucosal swabs were more easily performed and more consistently yielded *H parasuis*. Serotypes obtained from each site were the same.
- A lower level of colonization can be expected within the litters of young sows, and a low level of colonization by *H parasuis* at weaning likely predisposes piglets to clinical disease in the nursery, assuming the presence of a virulent serotype.
- More than one serotype of *H parasuis* can be present in the herd.
- Vaccination of sows did not influence the timing of apparent colonization by *H parasuis* or the serotypes prevalent within a litter.

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