An update on pathogenesis and control of *Salmonella* infections in pigs

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Pathogenesis of *Salmonella* infections in pigs

In order to be able to come up with efficient control measures to combat *Salmonella* infections in pigs, for example by means of vaccination, detailed knowledge of the pathogenesis should be the starting point.

Transmission of *Salmonella* between pigs is thought to occur mainly via the faecal–oral route. Depending on the inoculation dose and the used strain, experimental oral infection of pigs with *Salmonella* Typhimurium may result in clinical signs and faecal excretion of high numbers of bacteriaLoynachan and Harris, 2005 A.T. Loynachan and D.L. Harris, Dose determination for acute *Salmonella* infection in pigs, *Appl. Environ. Microbiol.* **71** (2005), pp. 2753–2755. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (5). During ingestion, *Salmonella* enters the tonsils in the soft palate and persists in the tonsillar crypts. The palatine tonsils are often heavily infected in pigs and should, therefore, not be underestimated as a source of *Salmonella* contamination during slaughter. Surprisingly little information has been gathered on how *Salmonella* interacts with and persists in the porcine tonsillar tissue. Persistence of *Salmonella* on the superficial epithelium of the tonsillar crypts has been reported (Van Parys et al., 2010). The mode of colonization of the tonsils is probably very different from the mechanism of colonization of the intestines (Boyen et al., 2006). Very recently, various genes that are expressed in the porcine tonsils during persistent infection have been identified using the *in vivo* expression technology (Van Parys et al., submitted). Bacteria that are swallowed and survive passage through the stomach, reach the gut. In the distal parts of the intestine, adherence to the intestinal mucosa is generally accepted to be the first step in the pathogenesis of *Salmonella* infections in pigs. It has recently been shown that in epithelial cells, reversible adhesion of *Salmonella* Typhimurium is mediated by type 1 fimbriae and irreversible adhesion (docking) is *Salmonella* Pathogenicity Island 1 (SPI-1) mediated (Misselwitz et al., 2011). Both type 1 fimbriae and SPI-1 have been shown to contribute to the colonization of the porcine intestinal tract. Following adhesion, *Salmonella* invades the intestinal epithelium. *Salmonella* Typhimurium can be found within the porcine enterocytes and mesenteric lymph nodes at 2 h after oral inoculation. Recently, it has been shown that the virulence genes encoded in the SPI-1 mediate this invasion step and that these genes are crucial for the colonization of the porcine gut and GALT. The rapid growth of *Salmonella* Typhimurium in the porcine gut and subsequent induction of pro-inflammatory responses may explain why pigs in most cases confine *Salmonella* Typhimurium infection to the intestines, whereas slow replication of *Salmonella* Choleraesuis may enable it to evade host immunity and subsequently spread beyond the intestinal boundariesPaulin et al., 2007 S.M. Paulin, A. Jagannathan, J. Campbell, T.S. Wallis and M.P. Stevens, Net replication of *Salmonella enterica* serovars Typhimurium and Choleraesuis in porcine intestinal mucosa and nodes is associated with their differential virulence, *Infect. Immun.* **75** (2007), pp. 3950–3960. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (15).

The systemic part of a *Salmonella* Typhimurium infection in pigs is not well-documented. It is generally accepted that *Salmonella* can spread throughout an organism using the blood stream or the lymphatic fluids and infect internal organs, although this has not yet been studied in detail in swine. The colonization of the gut associated lymphoid tissue (GALT), spleen and liver can result in prominent systemic and local immune responses. Macrophages are the cells of interest for *Salmonella* to disseminate to internal organs of different host species. The bacteria replicate rapidly intracellularly and cause the systemic phase of the infection, while interfering with the antibacterial mechanisms of the macrophages and inducing cell deathWaterman and Holden, 2003 S.R. Waterman and D.W. Holden, Functions and effectors of the *Salmonella* pathogenicity island 2 type III secretion system, *Cell. Microbiol.* **5** (2003), pp. 501–511. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (157). In pigs, non-typhoidal serotypes such as *Salmonella* Typhimurium, can reach liver and spleen shortly after experimental inoculation, but are cleared from these organs a few days after inoculation. At this time, the bacteria are still found in the gut, gut-associated lymph nodes and tonsils. These infections may result in long-term asymptomatic carriage of the bacterium. Since this carrier state in pigs is difficult to detect in live animals, either by bacteriological or serological methods, these pigs can bias monitoring programmes. Very few researchers have made an attempt to unravel the mechanism of the concealed, but prolonged infection in carrier pigs. Recent evidence suggests that *Salmonella* Typhimurium interferes with seroconversion in pigs, and that this phenomenon might be related to strain-dependent persistency capacities (Van Parys et al., 2011).

Stress-induced excretion of *Salmonella* Typhimurium by carrier pigs transported to the slaughterhouse may cause contamination of shipping equipment and holding areas, resulting in preslaughter transmission of *Salmonella* to non-infected pigs. Although the mechanism of this stress-induced excretion is not known, there are some indications that catecholamines and/or cortisol may play a role. It has been shown that *Salmonella* Typhimurium can “sense” catecholamines and as a result increase its growth rate. It has very recently been shown that the presence of cortisol has marked effects on the intracellular fate of *Salmonella* Typhimurium in porcine macrophages (Verbrugghe et al., 2011). The mechanism and the bacterial factors playing a role in this phenomenon are currently under investigation.

Designing control measures based on insights in the pathogenesis

Organic acids

The last few years, there is a widespread interest in “natural methods” to inhibit the spread of pathogenic bacteria in farm animals. Commercial preparations consisting of different kinds of organic acids not only appear to improve feed conversion and growth of animals, but also pathogen control has been reported, especially in poultry. Van Immerseel et al., 2004a F. Van Immerseel, J. De Buck, F. Boyen, L. Bohez, F. Pasmans, J. Volf, M. Sevcik, I. Rychlik, F. Haesebrouck and R. Ducatelle, Medium-chain fatty acids decrease colonization and invasion through *hilA* suppression shortly after infection of chickens with *Salmonella enterica* serovar Enteritidis, *Appl. Environ. Microbiol.* **70** (2004), pp. 3582–3587. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (35)The antibacterial effect of these products depends on the type of organic acid, the

bacterial species, the used concentration and the physical form through which it is administered to the animals. The composition of the currently used products is mostly empirically determined. Recent research suggests that *Salmonella* Typhimurium mainly uses two distinct sites and mechanisms for colonization of pigs, namely the tonsils on the one hand and the intestine and associated lymph nodes on the other hand. In order to combat *Salmonella* infections in pigs, measures that interfere with both tonsillar and intestinal colonization will probably yield the best results. Since the mechanisms of colonization of these sites seem to be different, the control measures should be designed accordingly.

Upper gastrointestinal tract

Contaminated feed is a well-known source for *Salmonella* introduction to the farm. The original concept of incorporating acids into the feed of poultry was based on the notion that the acids would decontaminate the feed itself and prevent *Salmonella* uptake. Even though no thorough research has been conducted concerning the control of a *Salmonella* infection at the tonsillar level, it seems likely that a similar effect could be achieved in the oral cavity. The type of acid and the concentration used would very important. The administration of acidified drinking water in pig farms has been reported to lower the prevalence of serologically positive pigs, even though this could not be confirmed in a recent controlled study (De Ridder et al., 2011). On the other hand, acid adaptation and acid tolerance genes have been described in *Salmonella* Typhimurium. Therefore, acidification of drinking water might even pre-condition *Salmonella* to survival in acid conditions, possibly reducing the effectiveness of the antibacterial barrier of the stomach.

Lower gastrointestinal tract

Orally administered organic acids are rapidly taken up by epithelial cells along the alimentary tract, thereby disappearing from the highly relevant lower parts of the gastrointestinal tract (ileum, caecum, colon). Therefore, researchers have attempted to transport the organic acids further down in the gastrointestinal tract by micro-encapsulation, which should prevent absorption of the acids in the upper tract. Certain short- and medium-chain fatty acids have been shown to decrease *Salmonella* invasion in enterocytes through the downregulation of SPI-1 encoded genes (Boyen et al., 2008). The concentrations of the acids necessary for this effect are below those necessary to exert a direct antimicrobial effect. Considering the importance of invasion in the colonization of the porcine gut, one could expect that any measure that interferes with this invasion step will decrease the bacterial load in the gut. Indeed, using coated butyric acid, we were able to lower intestinal colonization, spread between pigs and bacterial shedding in the faeces in independent and controlled studies (Boyen et al., 2008; De Ridder et al., 2011).

Vaccinating against *Salmonella* in pigs

Considering the positive effects of vaccination of laying hens on prevalence of *Salmonella* Enteritidis in eggs and *Salmonella* Enteritidis infections in humans, vaccination could also be a major tool to control *Salmonella* in pigs. The evidence available in scientific literature suggests that *Salmonella* vaccination is in fact associated with reduced *Salmonella* prevalence in swine at or near harvest. In addition, there are indications that even subclinical *Salmonella* infections can lead to weight gain losses in pigs indicating that vaccination to control salmonellosis in pigs may have an economic incentive for pig producers as well (Boyen et al., 2009; Farzan and Friendship, 2010).

Activating innate, mucosal, humoral and/or cellular immune response?

Vaccination might be able to reduce porcine carcass contamination and subsequently infections in humans in different ways, by interfering at different stages of the pathogenesis: inhibit early colonization, reduce excretion thereby lowering infection pressure at farm level, decrease spread between pigs by increasing the infective dose threshold, interfere with the development of the carrier state in the various target organs (gut, GALT and tonsils) and prevent the stress-related re-excretion at slaughter. To inhibit early colonization, adhesion to and/or invasion in epithelial intestinal cells should be blocked. This could probably be best achieved by attaining high levels of mucosal IgA. There are no indications which porcine immune response(s) are most important for reducing excretion shortly after infection, during the carrier state or in stress-related re-excretion periods. To interfere with the development of the carrier state, it can be expected that both the humoral and cellular immune response will be important. Probably innate, mucosal, humoral and cellular immunity all play a role in increasing the infective dose threshold.

Using live attenuated or inactivated vaccines for optimal effects?

At present, live vaccine strains are considered to offer a better protection against *Salmonella* infections compared to inactivated vaccines, probably due to the more pronounced cellular immune response and the induction of mucosal IgA production. Additional advantages of live vaccine strains are the possibility to administer these vaccines at a very young age, despite the presence of maternal antibodies, the flexibility of a live vaccine strain to switch to different colonization strategies at different target organs (gut-tonsils) and the possibility to administer it by mixing it in feed and/or water supply. The most difficult aspect of creating a good live attenuated vaccine is finding the perfect balance between attenuation to assure safety on the one hand and residual potency to assure the induction of a protective immune response on the other hand.
Bacterial attenuated vaccine strains can be divided in three types: (1) strains, which are attenuated without the attenuation being localised or characterised, (2) strains with mutations in genes that are important for the bacterial metabolism, for example auxotrophic mutant strains, (3) strains in which specific (virulence) genes were removed. The advantage of the latter group is that the vaccine strains are very well characterised and that reversion to the wild-type phenotype is extremely unlikely. Strains that lack one or more virulence genes important for clinical salmonellosis or for the induction of persistent infections in pigs might represent promising candidates for future vaccine development. Recent research has identified various virulence genes, playing a role in different stages of the pathogenesis of *Salmonella* Typhimurium infections in pigs. These findings may contribute to the development of more efficient and safer live attenuated vaccines.

Vaccinating sows or piglets?

Various researchers have reported that *Salmonella* prevalence at slaughter age is predominantly a result of infection during the finishing period, especially in multi-site herds. Therefore, currently, most control measures, including vaccination, are mainly focussed on older piglets and slaughter pigs. These strategies may indeed influence *Salmonella* prevalence and shedding at the end of the production cycle, but are not able to interfere with infection pressure and spread of the bacterium at younger stages of life. These early stages of the production cycle, can nevertheless be a crucial factor for the initial contamination of pig

batches, especially in pig farms where biosecurity and cleaning-disinfection protocols are good to excellent. It has been shown that early infection, occurring between birth and weaning, seemed to be a critical point for the *Salmonella* spread within a pig batch, and possibly within a herd and that sows may play an important role in the maintenance of *Salmonella* infections in farrow-to-finish herds (Nollet et al., 2005).

Interference of vaccination with *Salmonella* monitoring programmes

The purpose of monitoring and control programs is to reduce the risk of public health problems arising from the consumption of contaminated pork, reducing human disease and maintaining consumer confidence. Implementation of monitoring programs and coordination of control measures at harvest and post-harvest, are being used worldwide to prevent non-typhoidal *Salmonella* infections in humans from contaminated pork. Extensive national monitoring and control programs at the farm level (preharvest) are mostly conducted in the European countries. Most European monitoring programmes are based on serological screening, thus categorising the pig herds according to their assessed risk of carrying *Salmonella* into the slaughter plant Farmers with herds belonging to the category with the highest risk of introducing *Salmonella* into the slaughterhouse are assisted by the national governments to reduce the *Salmonella* load of their herd. There is currently one *Salmonella* vaccine registered for use in pigs in Europe which is a live attenuated vaccine. Even though this vaccine has promising features to decrease the *Salmonella* load on farm, the induced immune response interferes with the national control programmes of most of the European member states. A variant of this vaccine that enables differentiation of infected from vaccinated animals (DIVA) has been described (Selke et al., 2007). However, to differentiate vaccination with this DIVA strain from infection in the European monitoring systems, a new ELISA detection system should be implemented and validated in all European member states, which is very time consuming and expensive. A marker vaccine that would not interfere with the European (and/or other) control programmes would mean a huge step forward. Recent research by Leyman et al. (2011) has shown that the deletion of an LPS encoding gene might create a DIVA marker strain that would not interfere with the current European control programmes.

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