Review

Non-typhoidal Salmonella infections in pigs: A closer look at epidemiology, pathogenesis and control

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Abstract

Contaminated pork is an important source of Salmonella infections in humans. The increasing multiple antimicrobial resistance associated with pork-related serotypes such as Salmonella Typhimurium and Salmonella Derby may become a serious human health hazard in the near future. Governments try to anticipate the issue of non-typhoidal Salmonella infections in pork by starting monitoring programmes and coordinating control measures worldwide. A thorough knowledge of how these serotypes interact with the porcine host should form the basis for the development and optimisation of these monitoring and control programmes. During recent years, many researchers have focussed on different aspects of the pathogenesis of non-typhoidal Salmonella infections in pigs. The present manuscript reviews the importance of pigs and pork as a source for salmonellosis in humans and discusses commonly accepted and recent insights in the pathogenesis of non-typhoidal Salmonella infections in pigs, with emphasis on Salmonella Typhimurium, and to relate this knowledge to possible control measures.

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Keywords: Non-typhoidal Salmonella; Pig; Epidemiology; Pathogenesis; Control

Contents

1. Introduction .................................................................. 2
2. Pigs and pork as a source of salmonellosis in humans ........................................ 2
   2.1. Epidemiology ................................................................. 2
   2.2. Antimicrobial resistance .................................................. 4

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1. Introduction

*Salmonella* Choleraesuis was the first *Salmonella* serotype isolated from pigs (Salmon and Smith, 1886), only 2 years after the first isolation ever of *Salmonella*, performed by Gaffky in 1884 (Le Minor, 1994). In the course of time, more than 2400 different serotypes were isolated from different animal species, including pigs. *Salmonella* serotypes are differentiated based on their somatic and flagellar antigens. On the basis of the level of host restriction (Uzzau et al., 2000), *Salmonella* serotypes can also be classified as: (1) serotypes capable of causing a typhoid-like disease in a single host species (host-restricted serotypes, for example *Salmonella* Typhi in humans); (2) serotypes associated with one host species, but also able to cause disease in other hosts (host-adapted serotypes, for example *Salmonella* Choleraesuis and *Salmonella* Typhisuis in swine; Timoney et al., 1988; Selander et al., 1990) and (3) the vast majority of the remaining serotypes which rarely produce systemic infections but are able to colonize the alimentary tract of a wide range of animals (broad host range serotypes; for example *Salmonella* Typhimurium and *Salmonella* Derby; Fedorka-Cray et al., 2000). Infection of pigs with the swine adapted serotypes Typhisuis and Choleraesuis usually result in swine typhoid, characterized by severe systemic disease that is often fatal. This disease has been the subject of intense research (Gray et al., 1996; Lichtensteiger and Vimr, 2003; Chiu et al., 2004, 2005; Ku et al., 2005; Nishio et al., 2005; Zhao et al., 2006).

The pathogenesis in pigs of infection with broad-host range serotypes of *Salmonella* was largely neglected until recently. Although these infections may result in enteric and fatal systemic disease, infected pigs generally carry these serotypes asymptomatically in the tonsils, the intestines and the gut-associated lymphoid tissue (GALT) (Wood et al., 1989; Fedorka-Cray et al., 2000). Such carriers are a major reservoir of *Salmonella* and pose an important threat to animal and human health. A thorough knowledge of how these serotypes interact with the porcine host should form the basis for the development and evaluation of efficient monitoring programmes and control measures (Boyle et al., 2007). In the present review, the pathogenesis of infections with broad host range *Salmonella* serotypes is considered, with emphasis on *Salmonella* Typhimurium and, if relevant, in relation to common monitoring and control programs.

2. Pigs and pork as a source of salmonellosis in humans

2.1. Epidemiology

During 1950s and 1960s, *Salmonella* Choleraesuis, including variant Kunzendorf, was the pre-
dominant serotype isolated from pigs worldwide (Fedorka-Cray et al., 2000). At the present time, Salmonella Choleraesuis is still highly prevalent in North America and Asia, but is found rarely in Australia and Western European countries (Wilcock and Schwartz, 1992; Fedorka-Cray et al., 2000; Chiu et al., 2004; Davies et al., 2004; Chang et al., 2005; Nollet et al., 2006). Pigs can be infected by several Salmonella serotypes and the occurrence of these serotypes is also partly geographically determined (Fedorka-Cray et al., 2000; Loynachan et al., 2004).

All serotypes isolated from pigs are considered a hazard for public health by the European food safety authority (EFSA) (EFSA, 2006b). However, worldwide, the most commonly isolated non-typhoidal serotypes in pigs and pork are Salmonella Typhimurium, including variant Copenhagen, and Salmonella Derby (Letellier et al., 1999; Davies et al., 2004; Gebreyes et al., 2004; Valdezate et al., 2005; EFSA, 2006b; Rostagno et al., 2007).

Since its spectacular rise in the nineties until recently, Salmonella Enteritidis remained the most important serotype causing salmonellosis in humans in many countries. However, due to a remarkable drop in egg-related Salmonella Enteritidis infections in 2005 and 2006 in different European countries (Gillespie and Elson, 2005; Mossong et al., 2006; Collard et al., in press), Salmonella Typhimurium has now become the predominant serotype isolated from humans in Europe and pigs are probably the most important source of infection with this serotype in these countries. It has been estimated in various European countries that 15–23% of all cases of salmonellosis in humans, are related to the consumption of pork (Borch et al., 1996; Berends et al., 1998; Steinbach and Hartung, 1999; Van Pelt et al., 2000) and pork-related outbreaks of non-typhoidal salmonellosis in humans with fatal outcome have been described (Jansen et al., 2007). The relative importance of Salmonella contaminated pork as a cause of salmonellosis in humans in the European countries is likely to be even higher today, due to the decline in cases of disease caused by Salmonella Enteritidis. In USA, statistical models have predicted that every year approximately 100,000 human cases of salmonellosis are related to the consumption of pork, with a corresponding annual social cost of approximately 80 million dollar (Miller et al., 2005). In addition, the number of reported salmonellosis cases in humans is probably an underestimation of the true incidence by factors between 5 and 20 (Mead et al., 1999; Tschäpe and Bockemühl, 2002). Table 1 provides an overview of the prevalence of Salmonella in pork isolated in various countries.

Table 1
Salmonella isolated from pork in various countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Sampling</th>
<th>Sample</th>
<th>Sample All serotypes</th>
<th>Salmonella Typhimurium</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>1999</td>
<td>Post-harvest</td>
<td>Raw meat</td>
<td>209</td>
<td>3.3</td>
</tr>
<tr>
<td>Great Britain</td>
<td>1999</td>
<td>Harvest</td>
<td>Carcasses</td>
<td>2509</td>
<td>5.3</td>
</tr>
<tr>
<td>Vietnam</td>
<td>2000</td>
<td>Post-harvest</td>
<td>Raw meat</td>
<td>136</td>
<td>69.9</td>
</tr>
<tr>
<td>Mexico</td>
<td>2001</td>
<td>Post-harvest</td>
<td>Raw meat</td>
<td>339</td>
<td>58.1</td>
</tr>
<tr>
<td>Belgium</td>
<td>2004</td>
<td>Harvest</td>
<td>Raw meat</td>
<td>374</td>
<td>12.3</td>
</tr>
<tr>
<td>Denmark</td>
<td>2004</td>
<td>Harvest</td>
<td>Carcasses</td>
<td>34038</td>
<td>0.8</td>
</tr>
<tr>
<td>Finland</td>
<td>2004</td>
<td>Harvest</td>
<td>Carcasses</td>
<td>6576</td>
<td>0</td>
</tr>
<tr>
<td>Germany</td>
<td>2004</td>
<td>Harvest</td>
<td>Raw meat</td>
<td>4744</td>
<td>0.5</td>
</tr>
<tr>
<td>Hungary</td>
<td>2004</td>
<td>Harvest</td>
<td>Raw meat</td>
<td>8257</td>
<td>1.3</td>
</tr>
<tr>
<td>Italy</td>
<td>2004</td>
<td>Harvest</td>
<td>Raw meat</td>
<td>256</td>
<td>4.3</td>
</tr>
<tr>
<td>Italy</td>
<td>2004</td>
<td>Harvest</td>
<td>Carcasses</td>
<td>1096</td>
<td>3.6</td>
</tr>
<tr>
<td>Latvia</td>
<td>2004</td>
<td>Harvest</td>
<td>Raw meat</td>
<td>185</td>
<td>1.1</td>
</tr>
<tr>
<td>Malta</td>
<td>2004</td>
<td>Harvest</td>
<td>Raw meat</td>
<td>400</td>
<td>32.8</td>
</tr>
<tr>
<td>Poland</td>
<td>2004</td>
<td>Harvest</td>
<td>Raw meat</td>
<td>895</td>
<td>0.2</td>
</tr>
<tr>
<td>Portugal</td>
<td>2004</td>
<td>Harvest</td>
<td>Raw meat</td>
<td>256</td>
<td>15.2</td>
</tr>
<tr>
<td>Spain</td>
<td>2004</td>
<td>Harvest</td>
<td>Raw meat</td>
<td>147</td>
<td>10.2</td>
</tr>
</tbody>
</table>

* Data are from references Zhao et al. (2001), EFSA (2005), Davies et al. (2004), Phan et al. (2005), and Zaidi et al. (2006).
The public health risk of *Salmonella* infection from consumption of contaminated pork depends on multiple factors including the level of infection in the pig herd (Hill et al., 2003; Nollet et al., 2005), hygiene during carcass processing in the slaughterhouse (Borch et al., 1996), meat storage and distribution conditions (Mann et al., 2004) and finally the handling of undercooked pork by the consumer (Hill et al., 2003). As a first step in an integrated European approach on quantitative microbiological risk assessment (QMRA), the EFSA is preparing to carry out a QMRA on *Salmonella* in pigs, from the farm to the table, stressing the importance EFSA attributes to this topic (EFSA, 2006b; Hugas et al., 2007).

2.2. Antimicrobial resistance

Even though *Salmonella* infections in humans caused by non-typhoidal serotypes are usually self-limiting, effective antimicrobial therapy is essential if systemic spread occurs (Chiu et al., 1999; Su et al., 2004; Hsu et al., 2005; Alcaine et al., 2007). Systemic spread is frequently seen in individuals with immunodeficiency due to immaturity, senescence, chemotherapy, gastric hypoacidity, pregnancy or antecedent diseases. Together, individuals with these conditions constitute the so-called YOPI (young, old, pregnant, immunodeficient) segment of the population (Mossel et al., 2003). This group comprises 10–15% of the human population.

An obvious increase in antimicrobial resistance in non-typhoidal *Salmonella* serotypes has already been demonstrated in the early 1990s and has since then become a global problem (Su et al., 2004; Alcaine et al., 2007). The drug resistance rate, however, varies between different serotypes. *Salmonella* Enteritidis, for example, shows only limited acquired resistance compared to other important non-typhoidal serotypes (Su et al., 2004). The prevalence of acquired antimicrobial resistance is much higher in *Salmonella Typhimurium*. *Salmonella Typhimurium* strains belonging to the phage type DT104 are often found to be simultaneously resistant to 5 antimicrobial agents (ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines; Helms et al., 2005). Phage type DT104 is frequently isolated from pigs or pork (Threlfall, 2000; Gebreyes et al., 2004). Other phage types frequently isolated from pigs, like DT120 and DT193 are notoriously multidrug-resistant (Gebreyes et al., 2004). *Salmonella Typhimurium* strains resistant to 10 or more antimicrobial agents have been reported (Poppe et al., 2002).

Due to the spread of resistance to conventional antibiotics, extended spectrum cephalosporins and fluoroquinolones have become the drugs of choice to treat *Salmonella* infections in humans (Stoycheva and Murdjeva, 2006). Fluoroquinolones are also often used to treat the severe enteric fever form of salmonellosis in different animal species (Carter and Quinn, 2000; Jones, 2004; Hall and German, 2005). However, this has led to regular reports of resistance to these antibiotics as well, also in strains isolated from pigs and pork (Gazouli et al., 1998; Molbak et al., 1999; Baraniak et al., 2002; Chiu et al., 2002; Wang et al., 2006; Talavera-Rojas et al., 2007). Multidrug resistant *Salmonella Typhimurium* strains are also prevalent in antimicrobial free swine production systems, despite the absence of antimicrobial selection pressure (Thakur et al., 2007).

Clinical trials with new generation antimicrobials (azithromycin and gatifloxacin) that are effective against multiresistant *Salmonella* strains are underway (Boyle et al., 2007). Nevertheless, the increasing multiple antimicrobial resistance associated with pork-related serotypes such as *Salmonella Typhimurium* and *Salmonella Derby* may become a serious human health hazard in the near future (Hald et al., 2003; Akiba et al., 2006; Butaye et al., 2006; Vo et al., 2006; Alcaine et al., 2007). In addition, it has been reported that *Salmonella Choleraesuis* and *Salmonella Typhimurium* can generate hybrid plasmids, consisting of virulence genes on the one hand and of antimicrobial resistance genes on the other hand, possibly posing an even larger threat to public health (Chu and Chiu, 2006).

2.3. Current monitoring and control programs

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. *Salmonella* control measures can be implemented at three levels: the pre-harvest level (on farm), the harvest level (transport to and procedures in the
slaughterhouse) and the post-harvest level (cutting, processing, retail and food preparation at home).

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 (Mossel et al., 2003; Chen et al., 2006; Padungtod and Kaneene, 2006; EFSA, 2006b; Hamilton et al., 2007; Larsen et al., 2007; Rajic et al., 2007).

Extensive national monitoring and control programs at the farm level are mostly conducted in the European countries (regulation [EC] 2160/2003; Asai et al., 2002, 2006; EFSA, 2006b; Hamilton et al., 2007; Larsen et al., 2007; Rajic et al., 2007). Only the Scandinavian countries have been given low prevalence status by EFSA. In Sweden, preharvest and harvest monitoring programmes are being implemented on both a compulsory and a voluntary basis, using mainly bacteriological isolation to assess *Salmonella* contamination (Wahlström et al., 2000; EFSA, 2006b). The Danish, British, Irish and German programmes are based on serological testing of meat juice samples taken at the slaughterhouse, thus categorising the pig herds according to their assessed risk of carrying *Salmonella* into the slaughter plant (Nielsen et al., 2001; Davies et al., 2004; EFSA, 2006b; Merle et al., 2007). Belgian and Dutch monitoring programmes are similar, but the serological testing is currently performed on blood or serum samples collected on the farm (EFSA, 2006b; Bollaerts et al., 2007; Hanssen et al., 2007). 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 (EFSA, 2006a,b).

3. Pathogenesis of non-typhoidal *Salmonella* infections in pigs

3.1. Porcine colonization: to invade or not to invade . . .

3.1.1. Colonization of the upper gastrointestinal tract

Transmission of *Salmonella* between pigs is thought to occur mainly via the faecal–oral route. Depending on the inoculation dose, oral experimental infection of pigs with *Salmonella* Typhimurium may result in clinical signs and faecal excretion of high numbers of bacteria (Loynachan and Harris, 2005; Boyen et al., 2008a). Some studies showed that the upper respiratory tract and lungs may be a portal of entry as well (Fedorka-Cray et al., 1995; Proux et al., 2001) and in recent reports, it was found that airborne *Salmonella* Typhimurium transmission in weaned pigs over short distances is possible, but may be serotype-dependent (Oliveira et al., 2006, 2007). The pathogenesis of respiratory tract infections with *Salmonella* have, however, not been studied in detail and therefore, this review will focus on the oral infection route.

Porcine epithelial beta-defensin 1 is expressed in the dorsal tongue at antimicrobial concentrations and may contribute to the antimicrobial barrier properties of the dorsal tongue and oral epithelium (Shi et al., 1999). *Salmonellae* that overcome this barrier may colonize the tonsils. The palatine tonsils are often heavily infected in pigs and should, therefore, not be underestimated as a source of *Salmonella* contamination during slaughter (Wood et al., 1989; Kühnel and Blaha, 2004). During ingestion, *Salmonella* enters the tonsils in the soft palate and persists in the tonsillar crypts (Fedorka-Cray et al., 1995; Horter et al., 2003). Surprisingly, no detailed information has been gathered on how *Salmonella* interacts with and persists in the porcine tonsillar tissue, although some observations mention persistence of *Salmonella* on the superficial epithelium of the tonsillar crypts (Fedorka-Cray et al., 1995; Horter et al., 2003). The virulence factors used by *Salmonella* Typhimurium to colonize the tonsillar epithelium, probably without active invasion of tonsillar cells, are currently unknown. Indeed, a non-invasive strain was perfectly capable of colonizing the tonsils (Boyen et al., 2006c; Fig. 1). The mode of colonization of the tonsils may therefore be much different than the mechanism of colonization of the intestine.

Following ingestion, *Salmonella* must survive the low pH of the stomach. It has been shown that *Salmonellae* can adapt to and survive in acidic environments up to pH 3 by producing acid shock proteins (Audia et al., 2001; Smith, 2003; Berk et al., 2005). The non-glandular region and the cardiac gland zone of the porcine stomach have a pH range between 5 and 7 (Höller, 1970). Nevertheless, since the pH of
the fundus and pylorus of the porcine stomach in normal conditions decreases to pH 2 or even lower, many bacteria will be killed. When the pigs are fed a coarsely ground meal, this will result in a slow emptying of the stomach and consequently a longer stay in the acidic environment, reducing the number of surviving bacteria (Mikkelsen et al., 2004). In addition, it has been determined that the lethal effects of the porcine stomach contents are pH-dependent but that low pH is not the sole killing mechanism (Bearson et al., 2006).

3.1.2. Colonization of the lower gastrointestinal tract

Bacteria that survive passage through the stomach, travel to the small intestine where they encounter other antibacterial factors including bile salts, lysozyme and defensins. Even though Salmonella Typhimurium can be highly resistant against the direct antibacterial effects of bile salts (van Velkinburgh and Gunn, 1999), these salts repress the invasion of Salmonella in epithelial cells, possibly by decreasing virulence gene expression (Prouty and Gunn, 2000). Since high concentrations of bile salts are present in the upper part of the small intestine, this might explain why Salmonella preferentially colonizes the ileum, caecum and colon. Recently, it was shown that at least two types of defensins are present in the porcine small intestine, but their role in the pathogenesis of Salmonella Typhimurium infections in pigs is still unclear (Veldhuiizen et al., 2007).

In the distal parts of the intestine, adherence to the intestinal mucosa is generally accepted as the first step in the pathogenesis of Salmonella infections in pigs. Although multiple putative adhesins have been described for Salmonella Typhimurium, the type 1 fimbriae are the only ones which have been shown to contribute to the attachment to porcine enterocytes and subsequently the colonization of the intestinal tract (Althouse et al., 2003). In a recent signature-tagged mutagenesis assay, Salmonella atypical fimbriae (saf), located on Salmonella Pathogenicity Island 6 (SPI-6), were shown to play a role in porcine gut colonization, even though no statistically significant reduction was seen in the magnitude or duration of faecal excretion of a safA mutant (Carnell et al., 2007).

Following adhesion, Salmonella invades the intestinal epithelium. It has been shown that Salmonella can invade porcine absorptive enterocytes, M-cells and even goblet cells (Schauer et al., 2004). Salmonella Typhimurium is found within the porcine enterocytes and mesenteric lymph nodes at 2 h after oral inoculation (Reed et al., 1986). Intracellular bacteria were morphologically intact, and they were both free in the cytoplasm and membrane bound. Increased
epithelial cell loss has been observed during infection, as a result of caspase 3-dependent and independent programmed cell death in the proximal region of the jejunum (Schauser et al., 2005).

Recently, it has been shown that the virulence genes encoded in the Salmonella Pathogenicity Island 1 (SPI-1) mediate this invasion step and that these genes are crucial for the colonization of the gut and GALT (Boyen et al., 2006c; Brumme et al., 2007; Fig. 1). Using signature tagged mutagenesis, also several other virulence genes, including SPI-2 associated genes, have been identified as being important for the short-term colonization of the epithelium of the porcine gut (Carnell et al., 2007). An overview of all described virulence genes playing a role in the pathogenesis of Salmonella infections in pigs is given in Table 2.

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 boundaries (Paulin et al., 2007).

3.2. The mechanism of Salmonella-induced diarrhoea

Studies regarding the mechanisms involved in Salmonella Typhimurium-induced diarrhoea were mainly done in calf and mouse models (Tükel et al., 2006). Information on this topic in pigs is much more scarce.

When Salmonellae invade the intestinal epithelium, a phenomenon mediated by the SPI-1 type three secretion system (T3SS), the production of several cytokines, is induced in the porcine gut (Splichal et al., 2002; Cho and Chae, 2003; Utte et al., 2007; Volf et al., 2007). IL-8 is the most extensively studied, and for Salmonella Typhimurium-induced diarrhoea, probably the most important of these cytokines. Infection of porcine intestinal epithelial cells (Skjølaas et al., 2006; Volf et al., 2007) and porcine macrophages (Boyen et al., 2006a; Volf et al., 2007) with Salmonella Typhimurium increases IL-8 secretion by these cells. IL-8 is released from the basolateral aspect of infected epithelial cells and plays an important role in the initial movement of neutrophils (PMN) from the circulation into the subepithelial region (McCormick et al., 1995). The transepithelial migration of PMN into the lumen of the gut is mediated by the SPI-1 effector SipA. In human cell lines, the SipA protein induces the apical secretion of the pathogen elicited epithelial chemo-attractant (PEEC; Lee et al., 2000; Wall et al., 2007), which was later identified as the key regulator of mucosal inflammation, hepoxilin A3 (Mrsny et al., 2004). Recently, it has been shown that the T3SS of SPI-1 and the SPI-1 effector SipA are crucial in the recruitment of PMN to the porcine gut (Boyen et al., 2006c).

<table>
<thead>
<tr>
<th>Virulence gene</th>
<th>Role in pathogenesis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>FimA</td>
<td>Adhesion to intestinal epithelial cells</td>
<td>Althouse et al. (2003)</td>
</tr>
<tr>
<td>SafA</td>
<td>Attenuated in STM screening</td>
<td>Carnell et al. (2007)</td>
</tr>
<tr>
<td>SPI-1</td>
<td>Invasion in intestinal epithelial cells and development of enteritis</td>
<td>Boyen et al. (2006c), Brumme et al. (2007), Carnell et al. (2007)</td>
</tr>
<tr>
<td>SPI-2</td>
<td>Attenuated for causing disease and in STM screening</td>
<td>Carnell et al. (2007)</td>
</tr>
<tr>
<td>SugR</td>
<td>Attenuated in STM screening</td>
<td>Carnell et al. (2007)</td>
</tr>
<tr>
<td>MisL</td>
<td>Attenuated in STM screening</td>
<td>Carnell et al. (2007)</td>
</tr>
<tr>
<td>MgtC</td>
<td>Attenuated in STM screening</td>
<td>Carnell et al. (2007)</td>
</tr>
<tr>
<td>PipC</td>
<td>Attenuated in STM screening</td>
<td>Carnell et al. (2007)</td>
</tr>
<tr>
<td>Various plasmid-associated genes</td>
<td>Attenuated in STM screening</td>
<td>Carnell et al. (2007)</td>
</tr>
<tr>
<td>Various phage-associated genes</td>
<td>Attenuated in STM screening</td>
<td>Carnell et al. (2007)</td>
</tr>
<tr>
<td>Various envelope/surface structures</td>
<td>Attenuated in STM screening</td>
<td>Carnell et al. (2007)</td>
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<tr>
<td>Various house keeping genes</td>
<td>Attenuated in STM screening</td>
<td>Carnell et al. (2007)</td>
</tr>
<tr>
<td>Various hypothetical proteins</td>
<td>Attenuated in STM screening</td>
<td>Carnell et al. (2007)</td>
</tr>
</tbody>
</table>
In addition to the SPI-1-induced PMN influx, also other Salmonella-induced mechanisms leading to enteritis and/or diarrhoea have been described, although they are not yet confirmed in pig models. It has been shown that Salmonella can modulate the chloride secretion, contributing to the development of diarrhoea (Eckmann et al., 1997; Norris et al., 1998; Marcus et al., 2001; Zhou et al., 2001; Deleu et al., 2006). The SPI-1 effector SipB mediates macrophage death in the intestine by caspase-1 activation, causing the release of IL-1beta and IL-18, contributing to the inflammatory response (Hersh et al., 1999). Finally, the function of SopA and SopD is not fully understood, but both effectors also contribute to enteritis in a bovine intestinal loop model (Zhang et al., 2002; Bakowski et al., 2007).

The tools available for Salmonella to induce diarrhoea are overwhelming. Keeping this in mind, it may seem rather peculiar that most of the Salmonella Typhimurium infections in pigs are subclinical and asymptomatic. Apart from factors such as the infection pressure, the age and immunological status of the host, again Salmonella SPI-1 effectors may play a role. At least 3 SPI-1 effector proteins have been described that are able to down-regulate the transcription nuclear factor kappaB (NF-kB) and, subsequently, the host’s inflammatory responses: AvrA, a cysteine protease which inhibits pro-inflammatory cytokine production (Collier-Hyams et al., 2002; Ye et al., 2007); SspH1, a member of the family of T3SS effector proteins that share leucine-rich repeat motifs and are called Salmonella Translocated Effectors (STE’s; Miao et al., 1999; Haraga and Miller, 2006); and another STE, SptP, a protein reversing the SPI-1 effects concerning invasion (Haraga and Miller, 2003). Although these genes were first considered anti-virulence proteins, the lack of inflammatory response may help Salmonella to “sneak in” unnoticed. It has been shown that NF-kB-dependent gene expression is altered in mesenteric lymph nodes of pigs inoculated with Salmonella Typhimurium (Wang et al., 2007). Porcine GeneChip analysis and quantitative polymerase chain reaction analyses revealed transcriptional induction of NF-kB target genes at 24 h post-inoculation and suppression of the NF-kB pathway from 24 to 48 h post-inoculation, suggesting that the rapid, but transient induction of NF-kB pathways may allow the bacteria to enhance their survival chances (Wang et al., 2007).

The PMN in the gut belong to the first line of defense against a Salmonella infection. Inefficient uptake of Salmonella by PMN may provide an opportunity for the pathogen to colonize and/or replicate to levels that facilitate establishment of a carrier state or clinical infection in pigs (Stabel et al., 2002). Influx of PMN, however is a double-edged sword. On the one hand, the presence of high numbers of neutrophils in the porcine gut enables the host to overcome a Salmonella infection (Foster et al., 2003, 2005). On the other hand, the damage induced by activated neutrophils is considered the main cause of the gut pathology distinctive for Salmonella infections (Tükel et al., 2006).

3.3. Systemic spread: do macrophages matter?

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 swine. The colonization of the mesenteric lymph nodes, spleen and liver can result in prominent systemic and local immune responses (Dlabac et al., 1997). Macrophages are the cells of interest for host-restricted or -adapted Salmonella serotypes to disseminate to internal organs. 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 death (Waterman and Holden, 2003; Hueffer and Galan, 2004). The porcine immune system, however, differs in many aspects from that of other mammals (Scharek and Tedin, 2007). In pigs, sporadic Salmonella Typhimurium bacteria, present in liver and spleen shortly after experimental inoculation, do not seem to replicate and are even cleared from these organs a few days after inoculation (Boyen et al., 2008a). At this time, the bacteria are still found in the gut and gut-associated lymph nodes. Here, macrophages can be important players when it comes to long-term persistence.

Salmonella Pathogenicity Island 2 (SPI-2) is a prerequisite for Salmonella Typhimurium to survive in murine macrophages and for systemic spread in mice.
(Waterman and Holden, 2003). Brumme et al. (2007) showed that a SPI-2 deletion mutant strain was impaired in causing disease symptoms in pigs, but this deletion did not result in a significantly reduced colonization rate. Similar results were obtained by our research group (Boyen et al., 2008b).

Porcine monocytes and PMN respond in vitro to Salmonella Typhimurium with phagocytosis, oxidative burst and to some extent intracellular killing (Riber and Lind, 1999; Donné et al., 2005). Monocytes obtained from different pigs differed markedly in their reactive oxygen species (ROS) production and in their ability to kill the bacteria. Interestingly, high ROS production did not coincide with increased intracellular killing. No reactive nitrogen intermediates (RNI) production was detected by porcine PBM after stimulation with Salmonella Typhimurium or LPS (Donné et al., 2005). This is in agreement with other studies (Pampusch et al., 1998; Akunda et al., 2001) that suggested that NO synthase (iNOS) is not inducible in porcine immune cells with little or no upregulation following stimulation. Therefore, RNI production by iNOS does not appear to be an important component of the innate immune response to control intracellular Salmonella populations in pigs.

Both early and delayed cytotoxicity have been reported in porcine alveolar macrophages, but only the early cytotoxicity was SPI-1 dependent (Boyen et al., 2006a). It has also been shown that apoptosis-related genes are down-regulated in the mesenteric lymph nodes of Salmonella Typhimurium inoculated pigs at an early stage of infection, indicating that it might interfere with cell death signalling in pigs (Wang et al., 2007). The biological significance of Salmonella-induced cell death in the pathogenesis of Salmonella infections in pigs is not yet clear.

### 3.4. Persistent Salmonella Typhimurium infections in pigs

Infections of pigs with Salmonella Typhimurium may result in long-term asymptomatic carriage of these organisms (Wood et al., 1991). Since this carrier state in pigs is difficult to detect in live animals, either by bacteriological or serological methods (Baggesen and Wegener, 1993; Nollet et al., 2005), these pigs can bias monitoring programmes. 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 (Isaacson et al., 1999; Larsen et al., 2003; Boughton et al., 2007). Although the mechanism of this stress-induced excretion is not known, there are some indications that catecholamines may play a role. It has been shown that Salmonella Typhimurium can “sense” catecholamines and as a result increase its growth rate (Rahman et al., 2000; Williams et al., 2006; Methner et al., in press).

Very few researchers have made an attempt to unravel the mechanism of the concealed, but prolonged infection in carrier pigs (Boyen et al., 2006b; Wang et al., 2007). The CS54 Island has been characterized as an important locus for intestinal colonization and prolonged shedding in mice (Kingsley et al., 2000, 2003). The most important component of this island is ShdA, an outer membrane protein of the autotransporter family, which is expressed solely in the intestine. It has been shown, however, that a Salmonella Typhimurium shdA deletion mutant is not significantly impaired in persistence in pigs (Boyen et al., 2006b).

Recent insights in diverse areas of the pathogenesis, primarily described in mice, can also give us a clue which mechanisms might play a role in the persistence of Salmonella in pigs. The latest findings are changing our classical view of Salmonella as a fast growing intracellular pathogen and devastating bacterium. Research in the mouse model suggests that Salmonella may reduce its own intracellular growth rate (Cano et al., 2001; Jantsch et al., 2003; Sheppard et al., 2003; Monack et al., 2004) and may actively limit its impact on the infected tissues (Collier-Hyams et al., 2002; Haraga and Miller, 2003), as if it was a commensal (Tierrez and Garcia-del Portillo, 2005). It was found recently that Salmonella Typhimurium evades strong host responses by downregulating the local inflammatory response in pigs (Niewold et al., 2007; Wang et al., 2007).

In addition, Salmonella is able to interfere with the antigen presentation and the development of acquired immunity in mice (Mitchell et al., 2004; Qimron et al., 2004; Cheminay et al., 2005; Van der Velden et al., 2005; Alaniz et al., 2006; Luu et al., 2006). Also in pigs, it has been suggested that subversion of the dendritic cell function by Salmonella Typhimurium can prevent efficient stimulation of T-cell proliferation (Wang et al.,
Alternatively, it has been shown in mice that Salmonella Typhimurium does not replicate or cause cytotoxicity in fibroblasts, but remains in a persistent state (Martinez-Moya et al., 1998). Evidence for in vivo persistence in fibroblasts has been produced in mice (Garcia-del Portillo, 2006). Therefore, fibroblasts should not be neglected in the search for a mechanistic explanation for persistent infections in pigs. Even though the exact contribution of these mechanisms to the pathogenesis of the carrier state in pigs is not clear and that the porcine immune system differs in many aspects from that of mice (Scharek and Tedin, 2007), these findings in mice may shed new light on the mechanism of persistence in pigs in the future.

4. Interactions between Salmonella and other microbial agents

Various bacterial (Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae) and viral infections (hog cholera virus, porcine reproductive and respiratory syndrome virus (PRRSV), Aujeszyky’s disease virus) can result in immunodeficiency in pigs (Segales et al., 2004). Furthermore, some of these and also other swine pathogens (porcine parvovirus, swine influenza virus, African swine fever virus) are able to replicate in different immune cells and impair their function (Roth, 1999). These infections may lead to easier colonization by Salmonella, increased shedding or even higher mortality rates in pigs. For example, in utero infection with PRRSV inhibits phagocytosis of Salmonella in blood monocytes as well as the oxidative burst capacity of alveolar macrophages (Riber et al., 2004) and both pathogens may work synergistically to produce disease or persistence (Wills et al., 2000). The emergence of PRRSV in 1987 has been suggested to be a possible reason for the surge of Salmonella Choleraesuis infections in pigs in the USA the last few years (Chiu et al., 2004). PRRSV seropositivity during the fattening period also indicated an increased hazard for seroconversion for Salmonella (Beloeil et al., 2007).

Exposure to rumen protozoa led to enhancement of pathogenicity of Salmonella in a bovine infection model (Rasmussen et al., 2005). There are no reports describing enhanced virulence of Salmonella after exposure to other microbial agents in a porcine infection model. Interactions with intestinal nematodes may alter the excretion of Salmonella Typhimurium (Steenhard et al., 2002) or the development of enteritis symptoms (Arechavaleta et al., 1998). A dose dependency of the interaction was suggested. The relatively high number of parasites necessary to influence the Salmonella infection suggests that these common pig helminths generally do not influence the course of concurrent Salmonella Typhimurium infections under natural conditions (Steenhard et al., 2006). In contrast, the counts of Salmonella Typhimurium in the feces and in the cecal contents of piglets infected with both Salmonella Typhimurium and Isospora suis were significantly lower than in those infected with Salmonella Typhimurium alone (Baba and Gaafar, 1985). No mechanistic model was proposed by the authors to explain this phenomenon, however, the (subclinical) intestinal inflammation elicited by I. suis may aid in the limitation of Salmonella colonization (Foster et al., 2003).

5. Genetic resistance to Salmonella in swine

Although genetic-linked variance in immune responses is well known to occur in large domestic mammals such as pigs, specific resistance to Salmonella is less characterized (Wigley, 2004). The influence of swine major histocompatibility complex genes (SLA) on phagocytic and bactericidal activities of peripheral blood monocytes against Salmonella Typhimurium was measured in vitro using cultured cells (Lacey et al., 1989). Uptake and killing of Salmonella Typhimurium was highest in homozygous aa and cc haplotypes at 4 weeks and pigs with the c × d recombinant haplotype had highest uptake and killing of Salmonella Typhimurium at 8 weeks (Lacey et al., 1989).

The natural resistance-associated macrophage protein (SLC11a1; also called NRAMP1) has been reported to play a role in controlling the growth of several intracellular pathogens, including Salmonella (Roy and Malo, 2002). NRAMP1 has been identified and cloned in a number of domestic mammals including pigs (Sun et al., 1998; Zhang et al., 2000). In pigs NRAMP1 is strongly expressed on macrophages and neutrophils following stimulation with LPS (Zhang et al., 2000), and is upregulated significantly in the
mesenteric lymph nodes of pigs inoculated with Salmonella Typhimurium (Uthe et al., 2007; Wang et al., 2007).

A reference population of pigs bred to study resistance to Salmonella Choleraesuis infection indicated that a number of inherited immunological traits influence resistance to salmonellosis (Van Diemen et al., 2002). Neutrophils from the resistant animals showed increased phagocytic and antimicrobial activity and T-lymphocytes increased mitogen-induced proliferation, though no genes associated with this resistance were described. It would be presumptuous, however, to extrapolate these findings to non-typhoidal Salmonella serotypes.

Since the actual genes for resistance against Salmonella infections are not identified yet and since there are no pig lines that are less sensitive to non-typhoidal Salmonella infections, it is considered practically impossible to select for resistance in a breeding scheme (Velander et al., 2007).

6. Designing control measures based on the pathogenesis

6.1. 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,b, 2006). 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 (Boyen et al., 2006c; Huang et al., 2007). 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 important sites seem to be very different, the control measures should be designed accordingly.

6.1.1. Upper gastrointestinal tract

Contaminated feed is a well-known source for Salmonella introduction to the farm (Davies et al., 2004; Osterberg et al., 2006). 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 (Van Immerseel et al., 2006). 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 will be very important. (Van Immerseel et al., 2006).

The administration of acidified drinking water in pig farms has been shown to lower the prevalence of serologically positive pigs (van der Wolf et al., 2001). However, no experimental assays have been performed using acidified drinking water, nor has the effect on the colonization of the different organs been investigated. On the other hand, acid adaptation and acid tolerance genes have been described in Salmonella Typhimurium (Smith, 2003; Berk et al., 2005). Therefore, acidification of drinking water may precondition Salmonella to survival in acid conditions—possibly reducing the effectiveness of the antibacterial barrier of the stomach.

6.1.2. Lower gastrointestinal tract

Orally administered organic acids are rapidly taken up by epithelial cells along the alimentary tract, thereby disappearing in the highly relevant lower parts of the gastrointestinal tract. 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 (Van Immerseel et al., 2006). Certain short- and medium-chain fatty acids have been shown to decrease Salmonella invasion in enterocytes through the downregulation of SPI-1 encoded genes (Van Immerseel et al., 2004a; Gantois et al., 2006). The concentrations of the acids necessary for this effect are below those necessary to exert a direct antimicrobial effect (Gantois et al., 2006; Van Immerseel et al., 2004a). 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 and bacterial shedding in pigs (unpublished results) as has also been described in poultry (Van Immerseel et al., 2006).

6.2. Vaccination

Vaccination against zoonotic pathogens to prevent carriage is considered much more difficult than vaccination to prevent disease. It requires not only the induction of a local immune response to prevent mucosal colonization, it should also decrease or eliminate the presence of the bacteria at the farm-level to prevent cross-contamination at the slaughterhouse (Meeusen et al., 2007).

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 (Haesebrouck et al., 2004; Meeusen et al., 2007). 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. It has been suggested that virulence-gene deleted vaccines may be less immunogenic than metabolic-gene deleted vaccines (Meeusen et al., 2007). Nevertheless, it has been shown in pigs that protection against Salmonella Typhimurium was not dependent on T3SS secreted proteins of SPI-1 (Carnell et al., 2007).

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 vaccines. Such a vaccine should be able to: (1) prevent clinical symptoms, (2) reduce shedding by infected pigs and hence spreading to other pigs, (3) increase the threshold for infection of susceptible pigs and (4) induce a humoral response that is distinguishable of that induced during infection, not to interfere with monitoring programmes (Haesebrouck et al., 2004; Selke et al., 2007).

7. Concluding remarks

As stated above, it has been shown that Salmonella is able to interfere with the antigen presentation and the development of an immunological response in mice (Mitchell et al., 2004; Qimron et al., 2004). In practice, serologically negative swine herds are sometimes found to still produce pigs that are bacteriologically positive in the gut and associated lymph nodes at slaughter (Nollet et al., 2005). It has been suggested that these pigs were recently infected, so that the serological response was not fully developed at the time of sampling. However, if some Salmonella strains are truly able to actively decrease the immunological response, the current monitoring programmes, which usually are based on serology, may show inadequate in these cases. It is clear that more research in this area is needed.

Research on the pathogenesis of Salmonella infections in food producing animals remains an interesting and challenging topic. For relevant pathogenesis research, both host species and appropriate Salmonella strains should be chosen with care. Recent studies carried out in pigs improved our knowledge on mechanisms used by Salmonella Typhimurium to colonize the intestinal tract. On the other hand, the colonization of the tonsils remains largely unknown and merits further attention.

Presently acquired and future insights in the pathogenesis of Salmonella Typhimurium infections in pigs may aid in the fine-tuning of current control programmes and in developing more efficient and safer vaccines.

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