



HAL
open science

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

F. Boyen, F. Haesebrouck, D. Maes, F. van Immerseel, R. Ducatelle, F. Pasmans

► To cite this version:

F. Boyen, F. Haesebrouck, D. Maes, F. van Immerseel, R. Ducatelle, et al.. Non-typhoidal infections in pigs: a closer look at epidemiology, pathogenesis and control. *Veterinary Microbiology*, 2008, 130 (1-2), pp.1. 10.1016/j.vetmic.2007.12.017. hal-00532379

HAL Id: hal-00532379

<https://hal.science/hal-00532379>

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

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

Authors: F. Boyen, F. Haesebrouck, D. Maes, F. Van Immerseel, R. Ducatelle, F. Pasmans



PII: S0378-1135(08)00004-7
DOI: doi:10.1016/j.vetmic.2007.12.017
Reference: VETMIC 3935

To appear in: *VETMIC*

Received date: 29-10-2007
Revised date: 21-12-2007
Accepted date: 28-12-2007

Please cite this article as: Boyen, F., Haesebrouck, F., Maes, D., Van Immerseel, F., Ducatelle, R., Pasmans, F., Non-typhoidal *Salmonella* infections in pigs: a closer look at epidemiology, pathogenesis and control, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.12.017

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

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

Boyen^{1*} F., Haesebrouck¹ F., Maes² D., Van Immerseel¹ F., Ducatelle¹ R., Pasmans¹ F.

¹*Department of Pathology, Bacteriology and Avian Diseases
Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820
Merelbeke, Belgium*

²*Department of Reproduction, Obstetrics and Herd Health
Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820
Merelbeke, Belgium*

* Corresponding author: Tel. +32 9 2647359; Fax +32 9 2647494;

E-mail address: filip.boyen@UGent.be (F. Boyen)

26 **Abstract**

27

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

41

42 **Keywords**43 Non-typhoidal *Salmonella* – pig – epidemiology - pathogenesis – control

44 1. Introduction

45

46 *Salmonella* Choleraesuis was the first *Salmonella* serotype isolated from pigs (Salmon and
47 Smith, 1886), only 2 years after the first isolation ever of *Salmonella*, performed by Gaffky in
48 1884 (Le Minor, 1994). In the course of time, more than 2,400 different serotypes were
49 isolated from different animal species, including pigs. *Salmonella* serotypes are differentiated
50 based on their somatic and flagellar antigens. On the basis of the level of host restriction
51 (Uzzau et al., 2000), *Salmonella* serotypes can also be classified as: 1) serotypes capable of
52 causing a typhoid-like disease in a single host species (host restricted serotypes, for example
53 *Salmonella* Typhi in humans); 2) serotypes associated with one host species, but also able to
54 cause disease in other hosts (host adapted serotypes, for example *Salmonella* Choleraesuis
55 and *Salmonella* Typhisuis in swine; Timoney et al., 1988; Selander et al., 1990) and 3) the
56 vast majority of the remaining serotypes which rarely produce systemic infections but are able
57 to colonize the alimentary tract of a wide range of animals (broad host range serotypes; for
58 example *Salmonella* Typhimurium and *Salmonella* Derby; Fedorka-Cray et al., 2000).
59 Infection of pigs with the swine adapted serotypes Typhisuis and Choleraesuis usually result
60 in swine typhoid, characterized by severe systemic disease that is often fatal. This disease has
61 been the subject of intense research (Gray et al., 1996; Lichtensteiger and Vimr, 2003; Chiu et
62 al., 2004; Chiu et al., 2005; Ku et al., 2005; Nishio et al., 2005; Zhao et al. 2006).

63 The pathogenesis in pigs of infection with broad-host range serotypes of *Salmonella* was
64 largely neglected until recently. Although these infections may result in enteric and fatal
65 systemic disease, infected pigs generally carry these serotypes asymptotically in the
66 tonsils, the intestines and the gut-associated lymphoid tissue (GALT) (Wood et al., 1989;
67 Fedorka-Cray et al., 2000). Such carriers are a major reservoir of *Salmonella* and pose an
68 important threat to animal and human health. A thorough knowledge of how these serotypes

69 interact with the porcine host should form the basis for the development and evaluation of
70 efficient monitoring programmes and control measures (Boyle et al., 2007). In the present
71 review, the pathogenesis of infections with broad host range *Salmonella* serotypes is
72 considered, with emphasis on *Salmonella* Typhimurium and, if relevant, in relation to
73 common monitoring and control programs.

Accepted Manuscript

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

75

76 2.1. Epidemiology

77

78 During the 1950s and 1960s, *Salmonella* Choleraesuis, including variant Kunzendorf, was
79 the predominant serotype isolated from pigs worldwide (Fedorka-Cray et al., 2000). At the
80 present time, *Salmonella* Choleraesuis is still highly prevalent in North America and Asia, but
81 is found rarely in Australia and Western European countries (Wilcock and Schwartz, 1992;
82 Fedorka-Cray et al., 2000; Chiu et al., 2004; Davies et al., 2004; Chang et al., 2005; Nollet et
83 al., 2006). Pigs can be infected by several *Salmonella* serotypes and the occurrence of these
84 serotypes is also partly geographically determined (Fedorka-Cray et al., 2000; Loynachan et
85 al., 2004). All serotypes isolated from pigs are considered a hazard for public health by the
86 European food safety authority (EFSA) (EFSA, 2006b). However, worldwide, the most
87 commonly isolated non-typhoid serotypes in pigs and pork are *Salmonella* Typhimurium,
88 including variant Copenhagen, and *Salmonella* Derby (Letellier et al., 1999; Davies et al.,
89 2004; Gebreyes et al., 2004, Valdezate et al., 2005; EFSA, 2006b; Rostagno et al., 2007).

90 Since its spectacular rise in the nineties until recently, *Salmonella* Enteritidis remained the
91 most important serotype causing salmonellosis in humans in many countries. However, due to
92 a remarkable drop in egg-related *Salmonella* Enteritidis infections in 2005 and 2006 in
93 different European countries (Gillespie et al., 2005; Mossong et al., 2006; Collard et al.,
94 2007), *Salmonella* Typhimurium has now become the predominant serotype isolated from
95 humans in Europe and pigs are probably the most important source of infection with this
96 serotype in these countries. It has been estimated in various European countries that 15% to
97 23% of all cases of salmonellosis in humans, are related to the consumption of pork (Borch et
98 al., 1996; Berends et al., 1998; Steinbach and Hartung, 1999; Van Pelt et al., 2000) and pork-

99 related outbreaks of non-typhoid salmonellosis in humans with fatal outcome have been
100 described (Jansen et al., 2007). The relative importance of *Salmonella* contaminated pork as a
101 cause of salmonellosis in humans in the European countries is likely to be even higher today,
102 due to the decline in cases of disease caused by *Salmonella* Enteritidis. In the USA, statistical
103 models have predicted that every year approximately 100,000 human cases of salmonellosis
104 are related to the consumption of pork, with a corresponding annual social cost of
105 approximately 80 million dollar (Miller et al., 2005). In addition, the number of reported
106 salmonellosis cases in humans is probably an underestimation of the true incidence by factors
107 between 5 and 20 (Mead et al., 1999; Tschäpe and Bockemühl, 2002). Table 1 provides an
108 overview of the prevalence of *Salmonella* in pork isolated in various countries.

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

117

118 **2.2. Antimicrobial resistance**

119

120 Even though *Salmonella* infections in humans caused by non-typhoid serotypes are
121 usually self-limiting, effective antimicrobial therapy is essential if systemic spread occurs
122 (Chiu et al., 1999; Su et al., 2004; Hsu et al., 2005; Alcaine et al., 2007). Systemic spread is
123 frequently seen in individuals with immunodeficiency due to immaturity, senescence,

124 chemotherapy, gastric hypoacidity, pregnancy or antecedent diseases. Together, individuals
125 with these conditions constitute the so-called YOPI (young, old, pregnant, immunodeficient)
126 segment of the population (Mossel et al., 2003). This group comprises 10-15% of the human
127 population.

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

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

149 prevalent in antimicrobial free swine production systems, despite the absence of antimicrobial
150 selection pressure (Thakur et al., 2007).

151 Clinical trials with new generation antimicrobials (azithromycin and gatifloxacin) that are
152 effective against multiresistant *Salmonella* strains are underway (Boyle et al., 2007).
153 Nevertheless, the increasing multiple antimicrobial resistance associated with pork-related
154 serotypes such as *Salmonella* Typhimurium and *Salmonella* Derby may become a serious
155 human health hazard in the near future (Hald et al., 2003; Akiba et al., 2006; Butaye et al.,
156 2006; Vo et al., 2006; Alcaine et al., 2007). In addition, it has been reported that *Salmonella*
157 Choleraesuis and *Salmonella* Typhimurium can generate hybrid plasmids, consisting of
158 virulence genes on the one hand and of antimicrobial resistance genes on the other hand,
159 possibly posing an even larger threat to public health (Chu et al., 2006)

160

161 **2.3.Current monitoring and control programs**

162

163 The purpose of monitoring and control programs is to reduce the risk of public health
164 problems arising from the consumption of contaminated pork, reducing human disease and
165 maintaining consumer confidence. *Salmonella* control measures can be implemented at three
166 levels: the pre-harvest level (on farm), the harvest level (transport to and procedures in the
167 slaughterhouse) and the post-harvest level (cutting, processing, retail and food preparation at
168 home).

169 Implementation of monitoring programs and coordination of control measures at harvest
170 and post-harvest, are being used worldwide to prevent non-typhoidal *Salmonella* infections in
171 humans from contaminated pork (Mossel et al., 2003; Chen et al., 2006; Padungtod and
172 Kaneene, 2006; EFSA, 2006b; Hamilton et al. 2007; Larsen et al., 2007; Rajic et al., 2007).

173 Extensive national monitoring and control programs at the farm level are mostly
174 conducted in the European countries (regulation [EC] 2160/2003; Asai et al., 2002; Asai et
175 al., 2006; EFSA, 2006b; Hamilton et al. 2007; Larsen et al., 2007; Rajic et al., 2007). Only
176 the Scandinavian countries have been given low prevalence status by EFSA. In Sweden,
177 preharvest and harvest monitoring programmes are being implemented on both a compulsory
178 and a voluntary basis, using mainly bacteriological isolation to assess *Salmonella*
179 contamination (Wahlström et al., 2000; EFSA, 2006b). The Danish, British, Irish and German
180 programmes are based on serological testing of meat juice samples taken at the
181 slaughterhouse, thus categorising the pig herds according to their assessed risk of carrying
182 *Salmonella* into the slaughter plant (Nielsen et al., 2001; Davies et al., 2004; EFSA, 2006b;
183 Merle et al., 2007). Belgian and Dutch monitoring programmes are similar, but the serological
184 testing is currently performed on blood or serum samples collected on the farm (EFSA,
185 2006b; Bollaerts et al., 2007; Hanssen et al., 2007). Farmers with herds belonging to the
186 category with the highest risk of introducing *Salmonella* into the slaughterhouse are assisted
187 by the national governments to reduce the *Salmonella* load of their herd (EFSA, 2006a,b).

188

189 **3. Pathogenesis of non-typhoid *Salmonella* infections in pigs**

190

191 **3.1. Porcine colonization: to invade or not to invade...**

192

193 **3.1.1. Colonization of the Upper Gastrointestinal Tract**

194 Transmission of *Salmonella* between pigs is thought to occur mainly via the faecal-oral
195 route. Depending on the inoculation dose, oral experimental infection of pigs with *Salmonella*
196 Typhimurium may result in clinical signs and faecal excretion of high numbers of bacteria
197 (Loynachan et al., 2005; Boyen et al., 2007a). Some studies showed that the upper respiratory

198 tract and lungs may be a portal of entry as well (Fedorka-Cray et al., 1995; Proux et al., 2001)
199 and in recent reports, it was found that airborne *Salmonella* Typhimurium transmission in
200 weaned pigs over short distances is possible, but may be serotype-dependent (Oliveira et al.,
201 2006;2007). The pathogenesis of respiratory tract infections with *Salmonella* have, however,
202 not been studied in detail and therefore, this review will focus on the oral infection route.

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

218 Following ingestion, *Salmonella* must survive the low pH of the stomach. It has been
219 shown that salmonellae can adapt to and survive in acidic environments up to pH 3 by
220 producing acid shock proteins (Audia et al., 2001; Smith, 2003; Berk et al., 2005). The non-
221 glandular region and the cardiac gland zone of the porcine stomach have a pH range between
222 5 and 7 (Höller, 1970). Nevertheless, since the pH of the fundus and pylorus of the porcine

223 stomach in normal conditions decreases to pH 2 or even lower, many bacteria will be killed.
224 When the pigs are fed a coarsely ground meal, this will result in a slow emptying of the
225 stomach and consequently a longer stay in the acidic environment, reducing the number of
226 surviving bacteria (Mikkelsen et al., 2004). In addition, it has been determined that the lethal
227 effects of the porcine stomach contents are pH-dependent but that low pH is not the sole
228 killing mechanism (Bearson et al., 2006).

229

230 **3.1.2.Colonization of the Lower Gastrointestinal Tract**

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

241 In the distal parts of the intestine, adherence to the intestinal mucosa is generally accepted
242 as the first step in the pathogenesis of *Salmonella* infections in pigs. Although multiple
243 putative adhesins have been described for *Salmonella* Typhimurium, the type 1 fimbriae are
244 the only ones which have been shown to contribute to the attachment to porcine enterocytes
245 and subsequently the colonization of the intestinal tract (Althouse et al., 2003). In a recent
246 signature-tagged mutagenesis assay, *Salmonella* atypical fimbriae (*saf*), located on
247 *Salmonella* Pathogenicity Island 6 (SPI-6), were shown to play a role in porcine gut

248 colonization, even though no statistically significant reduction was seen in the magnitude or
249 duration of faecal excretion of a *safA* mutant (Carnell et al., 2007).

250 Following adhesion, *Salmonella* invades the intestinal epithelium. It has been shown that
251 *Salmonella* can invade porcine absorptive enterocytes, M-cells and even goblet cells
252 (Schauser et al., 2004). *Salmonella* Typhimurium is found within the porcine enterocytes and
253 mesenteric lymph nodes at 2 hours after oral inoculation (Reed et al., 1986). Intracellular
254 bacteria were morphologically intact, and they were both free in the cytoplasm and membrane
255 bound. Increased epithelial cell loss has been observed during infection, as a result of caspase
256 3 dependent and independent programmed cell death in the proximal region of the jejunum
257 (Schauser et al., 2005).

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

265 The rapid growth of *Salmonella* Typhimurium in the porcine gut and subsequent induction
266 of pro-inflammatory responses may explain why pigs in most cases confine *Salmonella*
267 Typhimurium infection to the intestines, whereas slow replication of *Salmonella* Choleraesuis
268 may enable it to evade host immunity and subsequently spread beyond the intestinal
269 boundaries (Paulin et al., 2007).

270

271 **3.2.The Mechanism of *Salmonella* Induced Diarrhoea**

272

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

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

291 In addition to the SPI-1 induced PMN influx, also other *Salmonella*-induced mechanisms
292 leading to enteritis and/or diarrhoea have been described, although they are not yet confirmed
293 in pig models. It has been shown that *Salmonella* can modulate the chloride secretion,
294 contributing to the development of diarrhoea (Eckmann et al., 1997; Norris et al., 1998;
295 Marcus et al., 2001; Zhou et al., 2001; Deleu et al., 2006). The SPI-1 effector SipB mediates
296 macrophage death in the intestine by caspase-1 activation, causing the release of IL-1beta and
297 IL-18, contributing to the inflammatory response (Hersh et al., 1999). Finally, the function of

298 SopA and SopD is not fully understood, but both effectors also contribute to enteritis in a
299 bovine intestinal loop model (Zhang et al., 2002; Bakowski et al., 2007).

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

319 The PMN in the gut belong to the first line of defense against a *Salmonella* infection.
320 Inefficient uptake of *Salmonella* by PMN may provide an opportunity for the pathogen to
321 colonize and/or replicate to levels that facilitate establishment of a carrier state or clinical
322 infection in pigs (Stabel et al., 2002). Influx of PMN, however is a double-edged sword. On

323 the one hand, the presence of high numbers of neutrophils in the porcine gut enables the host
324 to overcome a *Salmonella* infection (Foster et al., 2003,2005). On the other hand, the damage
325 induced by activated neutrophils is considered the main cause of the gut pathology distinctive
326 for *Salmonella* infections (Tükel et al., 2006).
327

Accepted Manuscript

328 3.3.Systemic Spread: Do Macrophages Matter?

329

330 The systemic part of a *Salmonella* Typhimurium infection in pigs is not well-documented.
331 It is generally accepted that *Salmonella* can spread throughout an organism using the blood
332 stream or the lymphatic fluids and infect internal organs, although this has not yet been
333 studied in swine. The colonization of the mesenteric lymph nodes, spleen and liver can result
334 in prominent systemic and local immune responses (Dlabac et al., 1997). Macrophages are the
335 cells of interest for host-restricted or -adapted *Salmonella* serotypes to disseminate to internal
336 organs. The bacteria replicate rapidly intracellularly and cause the systemic phase of the
337 infection, while interfering with the antibacterial mechanisms of the macrophages and
338 inducing cell death (Waterman and Holden, 2003; Hueffer and Galan, 2004). The porcine
339 immune system, however, differs in many aspects from that of other mammals (Scharek and
340 Tedin, 2007). In pigs, sporadic *Salmonella* Typhimurium bacteria, present in liver and spleen
341 shortly after experimental inoculation, do not seem to replicate and are even cleared from
342 these organs a few days after inoculation (Boyen et al., 2007a). At this time, the bacteria are
343 still found in the gut and gut-associated lymph nodes. Here, macrophages can be important
344 players when it comes to long term persistence.

345 *Salmonella* Pathogenicity Island 2 (SPI-2) is a prerequisite for *Salmonella* Typhimurium
346 to survive in murine macrophages and for systemic spread in mice (Waterman and Holden,
347 2003). Brumme et al. (2007) showed that a SPI-2 deletion mutant strain was impaired in
348 causing disease symptoms in pigs, but this deletion did not result in a significantly reduced
349 colonization rate. Similar results were obtained by our research group (Boyen et al., 2007b).

350 Porcine monocytes and PMN respond *in vitro* to *Salmonella* Typhimurium with
351 phagocytosis, oxidative burst and to some extent intracellular killing (Riber and Lind, 1999;
352 Donné et al., 2005). Monocytes obtained from different pigs differed markedly in their

353 reactive oxygen species (ROS) production and in their ability to kill the bacteria.
354 Interestingly, high ROS production did not coincide with increased intracellular killing. No
355 reactive nitrogen intermediates (RNI) production was detected by porcine PBM after
356 stimulation with *Salmonella* Typhimurium or LPS (Donné et al., 2005). This is in agreement
357 with other studies (Pampusch et al., 1998; Akunda et al., 2001) that suggested that NO
358 synthase (iNOS) is not inducible in porcine immune cells with little or no upregulation
359 following stimulation. Therefore, RNI production by iNOS does not appear to be an important
360 component of the innate immune response to control intracellular *Salmonella* populations in
361 pigs.

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

369

370 **3.4. Persistent *Salmonella* Typhimurium Infections in Pigs**

371

372 Infections of pigs with *Salmonella* Typhimurium may result in long-term asymptomatic
373 carriage of these organisms (Wood et al., 1991). Since this carrier state in pigs is difficult to
374 detect in live animals, either by bacteriological or serological methods (Baggesen and
375 Wegener, 1993; Nollet et al., 2005), these pigs can bias monitoring programmes. Stress-
376 induced excretion of *Salmonella* Typhimurium by carrier pigs transported to the
377 slaughterhouse may cause contamination of shipping equipment and holding areas, resulting

378 in preslaughter transmission of *Salmonella* to non-infected pigs (Isaacson et al., 1999; Larsen
379 et al., 2003; Boughton et al., 2007). Although the mechanism of this stress-induced excretion
380 is not known, there are some indications that catecholamines may play a role. It has been
381 shown that *Salmonella* Typhimurium can “sense” catecholamines and as a result increase its
382 growth rate (Rahman et al., 2000; Williams et al., 2006; Methner et al., 2007).

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

391 Recent insights in diverse areas of the pathogenesis, primarily described in mice, can also
392 give us a clue which mechanisms might play a role in the persistence of *Salmonella* in pigs.
393 The latest findings are changing our classical view of *Salmonella* as a fast growing
394 intracellular pathogen and devastating bacterium. Research in the mouse model suggests that
395 *Salmonella* may reduce its own intracellular growth rate (Cano et al., 2001; Jantsch et al.
396 2003; Sheppard et al., 2003; Monack et al., 2004) and may actively limit its impact on the
397 infected tissues (Collier-Hyams et al., 2002, Haraga and Miller, 2003), as if it was a
398 commensal (Tierrez and Garcia-del Portillo, 2005). It was found recently that *Salmonella*
399 Typhimurium evades strong host responses by downregulating the local inflammatory
400 response in pigs (Niewold et al., 2007; Wang, 2007).

401 In addition, *Salmonella* is able to interfere with the antigen presentation and the
402 development of acquired immunity in mice (Mitchell et al., 2004; Qimron et al., 2004,

403 Cheminay et al., 2005, Vandervelden et al., 2005; Alaniz et al., 2006; Luu et al., 2006). Also
404 in pigs, it has been suggested that subversion of the dendritic cell function by *Salmonella*
405 Typhimurium can prevent efficient stimulation of T-cell proliferation (Wang et al., 2007).
406 Alternatively, it has been shown in mice that *Salmonella* Typhimurium does not replicate or
407 cause cytotoxicity in fibroblasts, but remains in a persistent state (Martinez-Moya et al.,
408 1998). Evidence for *in vivo* persistence in fibroblasts has been produced in mice (Garcia-del
409 Portillo, 2006). Therefore, fibroblasts should not be neglected in the search for a mechanistic
410 explanation for persistent infections in pigs. Even though the exact contribution of these
411 mechanisms to the pathogenesis of the carrier state in pigs is not clear and that the porcine
412 immune system differs in many aspects from that of mice (Scharek and Tedin, 2007), these
413 findings in mice may shed a new light on the mechanism of persistence in pigs in the future.

414

415 **4. Interactions between *Salmonella* and other microbial agents**

416

417 Various bacterial (*Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*) and
418 viral infections (hog cholera virus, porcine reproductive and respiratory syndrome virus
419 (PRRSV), Aujeszky's disease virus) can result in immunodeficiency in pigs (Segales et al.,
420 2004). Furthermore, some of these and also other swine pathogens (porcine parvovirus, swine
421 influenza virus, African swine fever virus) are able to replicate in different immune cells and
422 impair their function (Roth, 1999). These infections may lead to easier colonization by
423 *Salmonella*, increased shedding or even higher mortality rates in pigs. For example, *in utero*
424 infection with PRRSV inhibits phagocytosis of *Salmonella* in blood monocytes as well as the
425 oxidative burst capacity of alveolar macrophages (Riber et al., 2004) and both pathogens may
426 work synergistically to produce disease or persistence (Wills et al., 2000). The emergence of
427 PRRSV in 1987 has been suggested to be a possible reason for the surge of *Salmonella*

428 Choleraesuis infections in pigs in the USA the last few years (Chiu et al., 2004). PRRSV
429 seropositivity during the fattening period also indicated an increased hazard for
430 seroconversion for *Salmonella* (Beloel et al., 2007).

431 Exposure to rumen protozoa led to enhancement of pathogenicity of *Salmonella* in a
432 bovine infection model (Rasmussen et al., 2005). There are no reports describing enhanced
433 virulence of *Salmonella* after exposure to other microbial agents in a porcine infection model.
434 Interactions with intestinal nematodes may alter the excretion of *Salmonella* Typhimurium
435 (Steenhard et al., 2002) or the development of enteritis symptoms (Arechavaleta et al., 1998).
436 A dose dependency of the interaction was suggested. The relatively high number of parasites
437 necessary to influence the *Salmonella* infection suggests that these common pig helminths
438 generally do not influence the course of concurrent *Salmonella* Typhimurium infections under
439 natural conditions (Steenhard et al., 2006). In contrast, the counts of *Salmonella* Typhimurium
440 in the feces and in the cecal contents of piglets infected with both *Salmonella* Typhimurium
441 and *Isoospora suis* were significantly lower than in those infected with *Salmonella*
442 Typhimurium alone (Baba and Gafaar, 1985). No mechanistic model was proposed by the
443 authors to explain this phenomenon, however, the (subclinical) intestinal inflammation
444 elicited by *Isoospora suis* may aid in the limitation of *Salmonella* colonization (Foster et al.,
445 2003).

446

447 **5. Genetic resistance to *Salmonella* in swine**

448

449 Although genetic-linked variance in immune responses is well known to occur in large
450 domestic mammals such as pigs, specific resistance to *Salmonella* is less characterized
451 (Wigley, 2004). The influence of swine major histocompatibility complex genes (SLA) on
452 phagocytic and bactericidal activities of peripheral blood monocytes against *Salmonella*

453 Typhimurium was measured *in vitro* using cultured cells (Lacey et al., 1989). Uptake and
454 killing of *Salmonella* Typhimurium was highest in homozygous aa and cc haplotypes at 4
455 weeks and pigs with the c x d recombinant haplotype had highest uptake and killing of
456 *Salmonella* Typhimurium at 8 weeks (Lacey et al., 1989).

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

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

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

475

476 6. Designing control measures based on the Pathogenesis

477

478 6.1.Organic acids

479

480 The last few years, there is a widespread interest in “natural methods” to inhibit the spread
481 of pathogenic bacteria in farm animals. Commercial preparations consisting of different kinds
482 of organic acids not only appear to improve feed conversion and growth of animals, but also
483 pathogen control has been reported, especially in poultry (Van Immerseel et al.,
484 2004a,b,2006) The antibacterial effect of these products depends on the type of organic acid,
485 the bacterial species, the used concentration and the physical form through which it is
486 administered to the animals. The composition of the currently used products is mostly
487 empirically determined.

488 Recent research suggests that *Salmonella* Typhimurium mainly uses two distinct sites and
489 mechanisms for colonization of pigs, namely the tonsils on the one hand and the intestine and
490 associated lymph nodes on the other hand (Boyen et al., 2006c; Huang et al., 2007). In order
491 to combat *Salmonella* infections in pigs, measures that interfere with both tonsillar and
492 intestinal colonization will probably yield the best results. Since the mechanisms of
493 colonization of these important sites seem to be very different, the control measures should be
494 designed accordingly.

495

496 6.1.1.Upper Gastrointestinal Tract

497 Contaminated feed is a well-known source for *Salmonella* introduction to the farm
498 (Davies et al., 2004; Osterberg et al., 2006). The original concept of incorporating acids into
499 the feed of poultry was based on the notion that the acids would decontaminate the feed itself
500 and prevent *Salmonella* uptake (Van Immerseel et al., 2006). Even though no thorough

501 research has been conducted concerning the control of a *Salmonella* infection at the tonsillar
502 level, it seems likely that a similar effect could be achieved in the oral cavity. The type of acid
503 and the concentration used will be very important. (Van Immerseel et al., 2006). The
504 administration of acidified drinking water in pig farms has been shown to lower the
505 prevalence of serologically positive pigs (van der Wolf et al., 2001). However, no
506 experimental assays have been performed using acidified drinking water, nor has the effect on
507 the colonization of the different organs been investigated. On the other hand, acid adaptation
508 and acid tolerance genes have been described in *Salmonella* Typhimurium (Smith et al., 2003;
509 Berk et al., 2005). Therefore, acidification of drinking water may pre-condition *Salmonella* to
510 survival in acid conditions- possibly reducing the effectiveness of the antibacterial barrier of
511 the stomach.

512

513 **6.1.2.Lower Gastrointestinal Tract**

514 Orally administered organic acids are rapidly taken up by epithelial cells along the
515 alimentary tract, thereby disappearing in the highly relevant lower parts of the gastro-
516 intestinal tract. Therefore, researchers have attempted to transport the organic acids further
517 down in the gastrointestinal tract by micro-encapsulation, which should prevent absorption of
518 the acids in the upper tract (Van Immerseel et al., 2006). Certain short- and medium-chain
519 fatty acids have been shown to decrease *Salmonella* invasion in enterocytes through the
520 downregulation of SPI-1 encoded genes (Van Immerseel et al., 2004a; Gantois et al., 2006).
521 The concentrations of the acids necessary for this effect are below those necessary to exert a
522 direct antimicrobial effect (Gantois et al., 2006; Van Immerseel et al., 2004a). Considering the
523 importance of invasion in the colonization of the porcine gut, one could expect that any
524 measure that interferes with this invasion step will decrease the bacterial load in the gut.
525 Indeed, using coated butyric acid, we were able to lower intestinal colonization and bacterial

526 shedding in pigs (unpublished results) as has also been described in poultry (Van Immerseel
527 et al., 2006).

528

529 **6.2. Vaccination**

530

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

536 At present, live vaccine strains are considered to offer a better protection against
537 *Salmonella* infections compared to inactivated vaccines, probably due to the more pronounced
538 cellular immune response and the induction of mucosal IgA production (Haesebrouck et al.,
539 2004; Meeusen et al., 2007). Bacterial attenuated vaccine strains can be divided in three
540 types: 1) strains, which are attenuated without the attenuation being localised or characterised,
541 2) strains with mutations in genes that are important for the bacterial metabolism, for example
542 auxotrophic mutant strains, 3) strains in which specific virulence genes were removed. The
543 advantage of the latter group is that the vaccine strains are very well characterised and that
544 reversion to the wild-type phenotype is extremely unlikely. Strains that lack one or more
545 virulence genes important for clinical salmonellosis or for the induction of persistent
546 infections in pigs might represent promising candidates for future vaccine development. It has
547 been suggested that virulence-gene deleted vaccines may be less immunogenic than
548 metabolic-gene deleted vaccines (Meeusen et al., 2007). Nevertheless, it has been shown in
549 pigs that protection against *Salmonella* Typhimurium was not dependent on T3SS secreted
550 proteins of SPI-1 (Carnell et al., 2007).

551 Recent research has identified various virulence genes, playing a role in different stages of
552 the pathogenesis of *Salmonella* Typhimurium infections in pigs. These findings may
553 contribute to the development of more efficient and safer vaccines. Such a vaccine should be
554 able to: (1) prevent clinical symptoms, (2) reduce shedding by infected pigs and hence
555 spreading to other pigs, (3) increase the threshold for infection of susceptible pigs and (4)
556 induce a humoral response that is distinguishable of that induced during infection, not to
557 interfere with monitoring programmes (Haesebrouck et al., 2004; Selke et al., 2007).

558

559 7. Concluding Remarks

560

561 As stated above, it has been shown that *Salmonella* is able to interfere with the antigen
562 presentation and the development of an immunological response in mice (Mitchell et al.,
563 2004; Qimron et al., 2004). In practice, serologically negative swine herds are sometimes
564 found to still produce pigs that are bacteriologically positive in the gut and associated lymph
565 nodes at slaughter (Nollet et al., 2005). It has been suggested that these pigs were recently
566 infected, so that the serological response was not fully developed at the time of sampling.
567 However, if some *Salmonella* strains are truly able to actively decrease the immunological
568 response, the current monitoring programmes, which usually are based on serology, may
569 show inadequate in these cases. It is clear that more research in this area is needed.

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

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

Accepted Manuscript

579 **References**

- 580 1. Althouse, C., Patterson, S., Fedorka-Cray, P., Isaacson R.E., 2003. Type 1 fimbriae of
581 *Salmonella enterica* serovar Typhimurium bind to enterocytes and contribute to
582 colonization of swine in vivo. *Infect. Immun.* 71, 6446-6452.
- 583 2. Akiba, M, Nakamura, K., Shinoda, D., Yoshii, N., Ito, H., Uchida, I., Nakazawa, M.,
584 2006. Detection and characterization of variant *Salmonella* genomic island 1s from
585 *Salmonella* Derby isolates. *Jpn. J. Infect. Dis.* 59, 341-345.
- 586 3. Akunda, J.K., Ahrens, F.A., Kramer, T.T., 2001. Evaluation of phagocytosis,
587 bactericidal activity, and production of superoxide anion, nitric oxide, and tumor
588 necrosis factor-alpha in Kupffer cells of neonatal pigs. *Am. J. Vet. Res.* 62, 1040-
589 1045.
- 590 4. Alaniz, R.C., Cummings, L.A., Bergman, M.A., Rassouljian-Barrett, S.L., Cookson,
591 B.T., 2006. *Salmonella* Typhimurium coordinately regulates FliC location and reduces
592 dendritic cell activation and antigen presentation to CD4+ T cells. *J. Immunol.* 177,
593 3983-3993.
- 594 5. Alcaine, S.D., Warnick L.D., Wiedmann, M., 2007. Antimicrobial resistance in
595 nontyphoidal *Salmonella*. *J. Food Prot.* 70, 780-790.
- 596 6. Arechavaleta, F., Molinari, J.L., Tato P., 1998. A *Taenia solium* metacestode factor
597 nonspecifically inhibits cytokine production. *Parasitol. Res.* 84, 117-122.
- 598 7. Asai, T., Esaki, H., Kojima, A., Ishihara, K., Tamura, Y., Takahashi, T., 2006.
599 Antimicrobial resistance in *Salmonella* isolates from apparently healthy food-
600 producing animal from 2000 to 2003: the first stage of Japanese veterinary
601 antimicrobial resistance monitoring (JVARM). *J. Vet. Med. Sci.* 68, 881-884.
- 602 8. Asai T, Fujii, S., Osumi, T., Otagiri, Y., Namimatsu, T., Sato, S., 2002. Isolation and
603 serological survey of *Salmonella* in pigs in Japan. *J. Vet. Med. Sci.* 64, 1011-1015.

- 604 9. Audia, J.P., Webb, C.C., Foster, J.W., 2001. Breaking through the acid barrier: an
605 orchestrated response to proton stress by enteric bacteria. *Int. J. Med. Microbiol.* 291,
606 97-106.
- 607 10. Baba, E., Gaafar, S.M., 1985. Interfering effect of *Isospora suis* infection on
608 *Salmonella* Typhimurium infection in swine. *Vet. Parasitol.* 17, 271-278.
- 609 11. Baggesen, D. L., Wegener, H.C., 1993. Detection of *Salmonella* in Swine. *Dansk. Vet.*
610 *Tidsskr.* 76, 1017-1022.
- 611 12. Bakowski M.A., Cirulis, J.T., Brown, N.F., Finlay, B.B., Brumell, J.H., 2007. SopD
612 acts cooperatively with SopB during *Salmonella enterica* serovar Typhimurium
613 invasion. *Cell. Microbiol.* In Press.
- 614 13. Baraniak, A., Sadowy, E., Hryniewicz, W., Gniadkowski, M., 2002. Two different
615 extended-spectrum beta-lactamases (ESBLs) in one of the first ESBL-producing
616 *Salmonella* isolates in Poland. *J. Clin. Microbiol.* 40, 1095-1097.
- 617 14. Bearson, S.M., Bearson, B.L., Rasmussen, M.A., 2006. Identification of *Salmonella*
618 *enterica* Serovar Typhimurium Genes Important for Survival in the Swine Gastric
619 Environment. *Appl. Environ. Microbiol.* 72, 2829-2836.
- 620 15. Beloeil, P.A., Chauvin, C., Proux, K., Fablet, C., Madec, F., Alioum A., 2007. Risk
621 factors for *Salmonella* seroconversion of fattening pigs in farrow-to-finish herds. *Vet.*
622 *Res.* 38, 835-848.
- 623 16. Berends, B.R., Van Knapen, F., Mossel, D.A.A., Burt, S.A., Snijders, J.M.A., 1998.
624 Impact on human health of *Salmonella* spp. On pork in The Netherlands and the
625 anticipated effects of some currently proposed control strategies. *Int. J. Food*
626 *Microbiol.* 44, 219-229.

- 627 17. Berk, P.A., Jonge, R., Zwietering, M.H., Abee, T., Kieboom, J., 2005. Acid resistance
628 variability among isolates of *Salmonella enterica* serovar Typhimurium DT104. J.
629 Appl. Microbiol. 99, 859-866.
- 630 18. Bollaerts, K., Aerts, M., Ribbens, S., Van Der Stede, Y., Boone, I., Mintiens, K.,
631 2007. Identification of *Salmonella* high risk pig farms in Belgium using semi-
632 parametric quantile regression. Proceedings of the 7th International Safepork
633 Symposium on the epidemiology & control of foodborne pathogens in pork, may 9-11,
634 Verona, Italy, pages 31-34.
- 635 19. Borch, E., Nesbakken, T., Christensen, H., 1996. Hazard identification in swine
636 slaughter with respect to foodborne bacteria. Int. J. Food Microbiol. 30, 9-25.
- 637 20. Boughton, C., Egan, J., Kelly, G., Markey, B., Leonard, N., 2007. Rapid infection of
638 pigs following exposure to environments contaminated with different levels of
639 *Salmonella typhimurium*. Foodborne Pathog. Dis. 4, 33-40.
- 640 21. Boyen, F., Pasmans, F., Donn , E., Van Immerseel, F., Adriaensen, C., Hernalsteens,
641 J.-P., Ducatelle, R., Haesebrouck, F., 2006a. Role of SPI-1 in the interactions of
642 *Salmonella* Typhimurium with porcine macrophages. Vet. Microbiol. 113, 35-44.
- 643 22. Boyen, F., Pasmans, F., Donn , E., Van Immerseel, F., Morgan, E., Adriaensen, C.,
644 Hernalsteens, J.-P., Wallis, T.S., Ducatelle, R., Haesebrouck, F., 2006b. The
645 fibronectin binding protein ShdA is not a prerequisite for long term faecal shedding of
646 *Salmonella* Typhimurium in pigs. Vet. Microbiol. 115, 284-290.
- 647 23. Boyen, F., Pasmans, F., Van Immerseel, F., Morgan, E., Adriaensen, C., Hernalsteens,
648 J.-P., Decostere, A., Ducatelle, R., Haesebrouck, F., 2006c. *Salmonella* Typhimurium
649 SPI-1 genes promote intestinal but not tonsillar colonization in pigs. Microb. Infect. 8,
650 2899-2907.

- 651 24. Boyen, F., Pasmans, F., Van Immerseel, F., Donné, E., Morgan, E., Ducatelle, R.,
652 Haesebrouck, F., 2007a. Porcine *in vitro* and *in vivo* models to assess the virulence of
653 *Salmonella enterica* serovar Typhimurium for pigs. Lab. Anim. Accepted for
654 publication.
- 655 25. Boyen, F., Pasmans, F., Van Immerseel, F., Morgan, E., Botteldoorn, N., Heyndrickx,
656 M., Volf, J., Favoreel, H., Hernalsteens, J.-P., Ducatelle, R., Haesebrouck, F., 2007b.
657 A limited role for SsrA/B in persistent *Salmonella* Typhimurium infections in pigs.
658 Vet. Mic., submitted.
- 659 26. Boyle, E.C., Bishop, J.L., Grassl, G.A., Finlay, B.B., 2007. *Salmonella*: from
660 pathogenesis to therapeutics. J. Bacteriol. 189, 1489-1495.
- 661 27. Brumme, S, Arnold, T., Sigmarsson, H., Lehmann, J., Scholz, H.C., Hardt, W.D.,
662 Hensel, A., Truyen, U. Roesler, U., 2007. Impact of *Salmonella* Typhimurium DT104
663 virulence factors *invC* and *sseD* on the onset, clinical course, colonization patterns and
664 immune response of porcine salmonellosis. Vet. Microbiol. 124, 274-285.
- 665 28. Butaye, P., Michael, G.B., Schwarz, S., Barrett, T.J., Brisabois, A., White, D.G., 2006.
666 The clonal spread of multidrug-resistant non-typhi *Salmonella* serotypes. Microbes
667 Infect. 8, 1891-1897.
- 668 29. Cano, D.A., Martinez-Moya, M., Pucciarelli, M.G., Groisman, E.A., Casadesus, J.,
669 Garcia-Del Portillo, F., 2001. *Salmonella enterica* serovar Typhimurium response
670 involved in attenuation of pathogen intracellular proliferation. Infect. Immun. 69,
671 6463-6474.
- 672 30. Carnell, S.C., Bowen, A., Morgan, E., Maskell, D.J., Wallis, T.S., Stevens, M.P.,
673 2007. Role in virulence and protective efficacy in pigs of *Salmonella enterica* serovar
674 Typhimurium secreted components identified by signature-tagged mutagenesis.
675 Microbiology. 153, 1940-1952.

- 676 31. Carter, M.E., Quinn, P.J., 2000. *Salmonella* infections in dogs and cats. In: Wray, C.,
677 Wray, A. (eds.), *Salmonella* in domestic animals, CAB International, Wallingford, p.
678 240.
- 679 32. Chang, C.C., Lin, Y.H., Chang, C.F., Yeh, K.S., Chiu, C.H., Chu, C., Chien, M.S.,
680 Hsu, Y.M., Tsai, L.S., Chiou, C.S., 2005. Epidemiologic relationship between
681 fluoroquinolone-resistant *Salmonella enterica* Serovar Choleraesuis strains isolated
682 from humans and pigs in Taiwan (1997 to 2002). *J. Clin. Microbiol.* 43, 2798-2804.
- 683 33. Cheminay, C., Mohlenbrink, A., Hensel, M., 2005. Intracellular *Salmonella* inhibit
684 antigen presentation by dendritic cells. *J. Immunol.* 174, 2892-2899.
- 685 34. Chen, T.H., Wang, Y.C., Chen, Y.T., Yang, C.H., Yeh, K.S., 2006. Serotype
686 occurrence and antimicrobial susceptibility of *Salmonella* isolates recovered from
687 pork carcasses in Taiwan (2000 through 2003). *J. Food Prot.* 69, 674-678.
- 688 35. Chiu, C. H., Lin, T.Y., Ou, J.T., 1999. Predictors for extraintestinal infections of non-
689 typhoidal *Salmonella* in patients without AIDS. *Int. J. Clin. Pract.* 53, 161-164
- 690 36. Chiu, C.H., Su, L.H., Chu, C., 2004. *Salmonella enterica* serotype Choleraesuis:
691 epidemiology, pathogenesis, clinical disease, and treatment. *Clin. Microbiol. Rev.*
692 17:311-322.
- 693 37. Chiu, C.H., Tang, P., Chu, C., Hu, S., Bao, Q., Yu, J., Chou, Y.Y., Wang, H.S., Lee,
694 Y.S., 2005. The genome sequence of *Salmonella enterica* serovar Choleraesuis, a
695 highly invasive and resistant zoonotic pathogen. *Nucleic Acids Res.* 33, 1690-1698.
- 696 38. Chiu, C.H., Wu, T.L., Su, L.H., Chu, C., Chia, J.H., Kuo, A.J., Chien, M.S., Lin, T.Y.,
697 2002. The emergence in Taiwan of fluoroquinolone resistance in *Salmonella enterica*
698 serotype Choleraesuis. *N. Engl. J. Med.* 346, 413-419.

- 699 39. Cho, W.S., Chae, C., 2003. Expression of inflammatory cytokines (TNF-alpha, IL-1,
700 IL-6 and IL-8) in colon of pigs naturally infected with *Salmonella* Typhimurium and
701 *Salmonella* Choleraesuis. J. Vet. Med. A Physiol. Pathol. Clin. Med. 50, 484-487.
- 702 40. Chu, C., Chiu, C.H., 2006. Evolution of the virulence plasmids of non-typhoid
703 *Salmonella* and its association with antimicrobial resistance. Microbes Infect. 8, 1931-
704 1936.
- 705 41. Collard, J.M., Bertrand, S., Dierick, K., Godard, C., Wildemaewe, C., Vermeersch, K.,
706 Duculot, J., Van Immerseel, F., Pasmans, F., Imberechts, H., Quinet, C., 2007. Drastic
707 decrease of human *Salmonella* Enteritidis in Belgium in 2005, shift in phage types and
708 influence on food-borne outbreaks. Epidemiol Infect. In Press.
- 709 42. Collier-Hyams, L.S., Zeng, H., Sun, J., Tomlinson, A.D., Bao, Z.Q., Chen, H.,
710 Madara, J.L., Orth, K., Neish, A.S., 2002. Cutting edge: *Salmonella* AvrA effector
711 inhibits the key proinflammatory, anti-apoptotic NF-kappa B pathway. J. Immunol.
712 169, 2846-2850.
- 713 43. Davies, P.R., Scott Hurd, H., Funk, J.A., Fedorka-Cray, P.J., Jones, F.T., 2004. The
714 role of contaminated feed in the epidemiology and control of *Salmonella enterica* in
715 pork production. Foodborne Pathog. Dis. 1, 202-215.
- 716 44. Deleu, S., Choi, K., Reece, J.M., Shears, S.B., 2006. Pathogenicity of *Salmonella*:
717 SopE-mediated membrane ruffling is independent of inositol phosphate signals. FEBS
718 Lett. 580, 1709-1715.
- 719 45. Dlabac, V., Trebichavsky, I., Rehakova, Z., Hofmanova, B., Splichal, I., Cukrowska,
720 B., 1997. Pathogenicity and protective effect of rough mutants of *Salmonella* species
721 in germ-free piglets. Infect. Immun. 65, 5238-5243.
- 722 46. Donné, E., Pasmans, F., Boyen, F., Van Immerseel, F., Adriaensen, C., Hernalsteens,
723 J.-P., Ducatelle, R., Haesebrouck, F., 2005. Survival of *Salmonella* serovar

- 724 Typhimurium inside porcine monocytes is associated with complement binding and
725 suppression of the production of reactive oxygen species. *Vet. Microbiol.* 107, 205-
726 214.
- 727 47. Eckmann, L., Rudolf, M.T., Ptasznik, A., Schultz, C., Jiang, T., Wolfson, N., Tsien,
728 R., Fierer, J., Shears, S.B., Kagnoff, M.F., Traynor-Kaplan, A.E., 1997. D-myo-
729 Inositol 1,4,5,6-tetrakisphosphate produced in human intestinal epithelial cells in
730 response to *Salmonella* invasion inhibits phosphoinositide 3-kinase signaling
731 pathways. *Proc. Natl. Acad. Sci. U.S.A.* 94, 14456-14460.
- 732 48. EFSA. 2005. The First Community Summary Report on Trends and Sources of
733 Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in
734 2004.
735 http://www.efsa.europa.eu/en/science/monitoring_zoonoses/reports/1277.html
- 736 49. EFSA. 2006a. The Second Community Summary Report on Trends and Sources of
737 Zoonoses, Zoonotic Agents, Antimicrobial resistance and Foodborne outbreaks in the
738 European Union in 2005
739 http://www.efsa.europa.eu/en/science/monitoring_zoonoses/reports/zoonoses_report_2
740 005.html
- 741 50. EFSA. 2006b. Opinion of the Scientific Panel on Biological Hazards on the request
742 from the Commission related to “Risk assessment and mitigation options of
743 *Salmonella* in pig production”. *The EFSA Journal* 341. 1-131
- 744 51. Fedorka-Cray, P.J., Gray, J.T., Wray, C., 2000. *Salmonella* infections in pigs. In:
745 Wray, C., Wray, A. (eds.), *Salmonella* in domestic animals, CAB International,
746 Wallingford, pp. 191-207.

- 747 52. Fedorka-Cray, P.J., Kelley, L.C., Stabel, T.J., Gray, J.T., Laufer, J.A., 1995. Alternate
748 routes of invasion may affect pathogenesis of *Salmonella* Typhimurium in swine.
749 Infect. Immun. 63, 2658-2664.
- 750 53. Foster, N., Hulme, S., Lovell, M., Reed, K., Barrow, P., 2005. Stimulation of gp91
751 phagocytic oxidase and reactive oxygen species in neutrophils by an avirulent
752 *Salmonella enterica* serovar infantis strain protects gnotobiotic piglets from lethal
753 challenge with serovar Typhimurium strain F98 without inducing intestinal pathology.
754 Infect. Immun. 73, 4539-4547.
- 755 54. Foster, N., Lovell, M.A., Marston, K.L., Hulme, S.D., Frost, A.J., Bland, P., Barrow,
756 P., 2003. Rapid protection of gnotobiotic pigs against experimental salmonellosis
757 following induction of polymorphonuclear leukocytes by avirulent *Salmonella*
758 *enterica*. Infect. Immun. 71, 2182-2191.
- 759 55. Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Hautefort, I., Thompson, A.,
760 Hinton, J.C., Van Immerseel, F., 2006. Butyrate specifically down-regulates
761 *Salmonella* pathogenicity island 1 gene expression. Appl. Environ. Microbiol. 72,
762 946-949.
- 763 56. Garcia-del Portillo, F. 2006. The unique lifestyle of *Salmonella* in fibroblast cells. The
764 proceedings of ASM conference *Salmonella*: from pathogenesis to therapeutics.
765 September 9-16, Victoria, Canada, p 20.
- 766 57. Gazouli, M., Tzelepi, E., Markogiannakis, A., Legakis, N.J., Tzouvelekis, L.S., 1998.
767 Two novel plasmid-mediated cefotaxime-hydrolyzing beta-lactamases (CTX-M-5 and
768 CTX-M-6) from *Salmonella* Typhimurium. FEMS Microbiol. Lett. 165, 289-293.
- 769 58. Gebreyes, W.A., Thakur, S., Davies, P.R., Funk, J.A., Altier, C., 2004. Trends in
770 antimicrobial resistance, phage types and integrons among *Salmonella* serotypes from
771 pigs, 1997-2000. J. Antimicrob. Chemother. 53, 997-1003.

- 772 59. Gillespie, I., Elson, R., 2005. Successful reduction of human *Salmonella* Enteritidis
773 infection in England and Wales. *Eurosurveillance* 2005; 10, issue 11.
774 <http://www.eurosurveillance.org/ew/2005/051117.asp#2>
- 775 60. Gray, J.T., Stabel, T.J., Fedorka-Cray, P.J., 1996. Effect of dose on the immune
776 response and persistence of *Salmonella choleraesuis* infection in swine. *Am. J. Vet.*
777 *Res.* 57, 313-319.
- 778 61. Haesebrouck, F., Pasmans, F., Chiers, K., Maes, D., Ducatelle, R., Decostere, A.,
779 2004. Efficacy of vaccines against bacterial diseases in swine: what can we expect?
780 *Vet. Microbiol.* 100, 255-268.
- 781 62. Hald, T., Wingstrand, A., Swanenburg, M., von Altrock, A., Thorberg, B.M., 2003.
782 The occurrence and epidemiology of *Salmonella* in European pig slaughterhouses.
783 *Epidemiol. Infect.* 131, 1187-1203.
- 784 63. Hall, E.J., German, A.J., 2005. Diseases of the small intestine. In: Ettinger S.J.,
785 Feldman E.C. (eds.), *Textbook of Veterinary Internal Medicine: Diseases of the Dog*
786 *and Cat*, sixth edition, Elsevier Saunders, St. Louis, p. 1356.
- 787 64. Hamilton, D.R., Smith, P., Pointon, A., 2007. National *Salmonella* and *E. coli*
788 monitoring (ESAM) data from Australian pig carcasses from 2000 to 2006.
789 Proceedings of the 7th International Safepork Symposium on the epidemiology &
790 control of foodborne pathogens in pork, may 9-11, Verona, Italy, pages 129-132.
- 791 65. Hanssen, E.J., Swanenburg, M., Maassen, C.B.M., 2007. The dutch *Salmonella*
792 monitoring programme for pigs and some recommendations for control plans in the
793 future. Proceedings of the 7th International Safepork Symposium on the epidemiology
794 & control of foodborne pathogens in pork, may 9-11, Verona, Italy, pages 169-172.

- 795 66. Haraga, A., Miller, S.I., 2003. A *Salmonella enterica* serovar Typhimurium
796 translocated leucine-rich repeat effector protein inhibits NF-kappa B-dependent gene
797 expression. *Infect. Immun.* 71, 4052-4058.
- 798 67. Haraga, A., Miller, S.I., 2006. *Salmonella* type III secretion effector interacts with the
799 mammalian serine/threonine protein kinase PKN1. *Cell. Microbiol.* 8, 837-846.
- 800 68. Helms, M., Ethelberg, S., Molbak, K., 2005. DT104 Study Group. International
801 *Salmonella* Typhimurium DT104 infections, 1992-2001. *Emerg. Infect. Dis.* 11, 859-
802 867.
- 803 69. Hersh, D., Monack, D.M., Smith, M.R., Ghori, N., Falkow, S., Zychlinsky, A., 1999.
804 The *Salmonella* invasin SipB induces macrophage apoptosis by binding to caspase-1.
805 *Proc. Natl. Acad. Sci. U.S.A.* 96, 2396-2401.
- 806 70. Hill, A., England, T., Snary, E., Cook, A., Kelly, L., Evans, S., Wouldridge, M., 2003.
807 A “farm-to-consumption” risk assessment for *Salmonella* Typhimurium in pigs.
808 Department of risk research. Veterinary Laboratories Agency-Weybridge.
- 809 71. Höller, H. 1970. Studies on the secretion of the cardiac zone in the pig stomach.
810 *Zentralbl. Veterinärmed. A.* 17, 857-873.
- 811 72. Horter, D.C., Yoon, K.J., Zimmerman, J.J., 2003. A review of porcine tonsils in
812 immunity and disease. *Anim. Health Res. Rev.* 4, 143-155.
- 813 73. Hsu, R.B., Lin, F.Y., Chen, R.J., Hsueh, P.R., Wang, S.S., 2005. Antimicrobial drug
814 resistance in *Salmonella*-infected aortic aneurysms. *Ann. Thorac. Surg.* 80, 530-536.
- 815 74. Huang, Y., Leming, C.L., Suyemoto, M., Altier, C., 2007. Genome-wide screen of
816 *Salmonella* genes expressed during pig infection using in vivo expression technology.
817 *Appl. Environ. Microbiol.* In Press.
- 818 75. Hueffer, K., Galan, J.E., 2004. *Salmonella*-induced macrophage death: multiple
819 mechanisms, different outcomes. *Cell. Microbiol.* 6, 1019-1025.

- 820 76. Hugas, M., Tsigarida, E., Robinson, T., Calistri, P., 2007. Risk assessment of
821 biological hazards in the European Union. *Int. J. Food Microbiol.* In Press.
- 822 77. Isaacson, R.E., Firkins, L.D., Weigel, R.M., Zuckermann, F.A., DiPietro, J.A., 1999.
823 Effect of transportation and feed withdrawal on shedding of *Salmonella* Typhimurium
824 among experimentally infected pigs. *Am. J. Vet. Res.* 60, 1155-1158.
- 825 78. Jansen, A., Frank, C., Stark, K., 2007. Pork and pork products as a source for human
826 salmonellosis in Germany. *Berl. Munch. Tierarztl. Wochenschr.* 120, 340-346.
- 827 79. Jantsch, J., Cheminay, C., Chakravorty, D., Lindig, T., Hein, J., Hensel, M., 2003.
828 Intracellular activities of *Salmonella enterica* in murine dendritic cells. *Cell.*
829 *Microbiol.* 5, 933-945.
- 830 80. Jones, S.L. 2004. Inflammatory diseases of the gastrointestinal tract causing diarrhea.
831 In: Reed S.M., Warwick M.B., Sellon D.C. (eds.), *Equine Internal Medicine*, second
832 edition, Elsevier Saunders, St. Louis, p. 904.
- 833 81. Kingsley, R.A., Humphries, A.D., Weening, E.H., de Zoete, M.R., Winter, S.,
834 Papaconstantinou, A., Dougan G., Bäumler, A.J., 2003. Molecular and
835 phenotypic analysis of the CS54 island of *Salmonella enterica* serotype Typhimurium:
836 identification of intestinal colonization and persistence determinants. *Infect. Immun.*
837 71, 629-640.
- 838 82. Kingsley, R.A., van Amsterdam, K., Kramer, N., Bäumler, A.J., The *shdA* gene is
839 restricted to serotypes of *Salmonella enterica subspecies I* and contributes to efficient
840 and prolonged faecal shedding. *Infect. Immun.* 68, 2720-2727.
- 841 83. Ku, Y.W., McDonough, S.P., Palaniappan, R.U., Chang, C.F., Chang, Y.F., 2005.
842 Novel attenuated *Salmonella enterica* serovar Choleraesuis strains as live vaccine
843 candidates generated by signature-tagged mutagenesis. *Infect. Immun.* 73, 8194-8203.

- 844 84. Kühnel, K., Blaha, Th., 2004. Investigations on targeted intervention measures for
845 minimizing *Salmonella* cross-contamination during slaughter. Proceedings of the 18th
846 IPVS Congress, Hamburg, Germany, 651.
- 847 85. Lacey, C., Wilkie, B.N., Kennedy, B.W., Mallard, B.A., 1989. Genetic and other
848 effects on bacterial phagocytosis and killing by cultured peripheral blood monocytes
849 of SLA-defined miniature pigs. *Anim. Genet.* 20, 371-381.
- 850 86. Larsen, S.T., McKean, J.D., Hurd, H.S., Rostagno, M.H., Griffith, R.W., Wesley, I.V.,
851 2003. Impact of commercial preharvest transportation and holding on the prevalence
852 of *Salmonella enterica* in cull sows. *J. Food Prot.* 66, 1134-1138.
- 853 87. Larsen, S., Sundberg, P., Wagstrom, E., Niekamp, S., Risa, E., 2007. Pork Quality
854 Assurance PlusTM Program. Proceedings of the 7th International Safepork Symposium
855 on the epidemiology & control of foodborne pathogens in pork, may 9-11, Verona,
856 Italy, pages 137-139.
- 857 88. Lee, C.A., Silva, M., Siber, A.M., Kelly, A.J., Galyov, E., McCormick, B.A., 2000. A
858 secreted *Salmonella* protein induces a proinflammatory response in epithelial cells,
859 which promotes neutrophil migration. *Proc. Natl. Acad. Sci. U.S.A.* 97, 12283-12288.
- 860 89. Le Minor, L. 1994. The genus *Salmonella*. In: Balloxs A., Trüper HG, Dworkin M,
861 Harber W, Scheiffer HK (eds) *The prokaryotes*. Springer, New York, 2760-2774.
- 862 90. Letellier, A, Messier, S., Pare, J., Menard, J., Quessy, S., 1999. Distribution of
863 *Salmonella* in swine herds in Quebec. *Vet. Microbiol.* 67, 299-306.
- 864 91. Lichtensteiger, C.A., Vimr, E.R., 2003. Systemic and enteric colonization of pigs by a
865 *hilA* signature-tagged mutant of *Salmonella choleraesuis*. *Microb. Pathog.* 34, 149-
866 154.
- 867 92. Loynachan, A.T., Harris, D.L., 2005. Dose determination for acute *Salmonella*
868 infection in pigs. *Appl. Environ. Microbiol.* 71, 2753-2755.

- 869 93. Loynachan, A.T., Nugent, J.M., Erdman, M.M., Harris, D.L., 2004. Acute infection of
870 swine by various *Salmonella* serovars. *J. Food Prot.* 67, 1484-1488.
- 871 94. Luu, R.A., Gurnani, K., Dudani, R., Kammara, R., van Faassen, H., Sirard, J.C.,
872 Krishnan, L., Sad, S., 2006. Delayed expansion and contraction of CD8⁺ T cell
873 response during infection with virulent *Salmonella* Typhimurium. *J. Immunol.* 177,
874 1516-1525.
- 875 95. Mann, J.E., Smith, L., Brashears, M.M., 2004. Validation of time and temperature
876 values as critical limits for *Salmonella* and background flora growth during the
877 production of fresh ground and boneless pork products. *J. Food Prot.* 67, 1389-1393.
- 878 96. Marcus, S.L., Wenk, M.R., Steele-Mortimer, O., Finlay, B.B., 2001. A synaptojanin-
879 homologous region of *Salmonella* Typhimurium SigD is essential for inositol
880 phosphatase activity and Akt activation. *FEBS Lett.* 494, 201-207.
- 881 97. Martinez-Moya M., de Pedro, M.A., Schwarz, H., Garcia-del Portillo, F., 1998.
882 Inhibition of *Salmonella* intracellular proliferation by non-phagocytic eucaryotic cells.
883 *Res. Microbiol.* 149, 309-318.
- 884 98. McCormick, B.A., Hofman, P.M., Kim, J., Carnes, D.K., Miller, S.I., Madara, J.L.,
885 1995. Surface attachment of *Salmonella* Typhimurium to intestinal epithelia imprints
886 the subepithelial matrix with gradients chemotactic for neutrophils. *J. Cell. Biol.* 131,
887 1599-1608.
- 888 99. Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresse, J.S., Shapiro, C., Griffin,
889 P.M., Tauxe, R.V., 1999. Food-related illness and death in the United States. *Emerg.*
890 *Infect. Dis.* 5, 607-625.
- 891 100. Meeusen, E.N., Walker, J., Peters, A., Pastoret, P.P., Jungersen, G., 2007.
892 Current status of veterinary vaccines. *Clin. Microbiol. Rev.* 20, 489-510.

- 893 101. Merle, R., Schneider, B., Franz, B., Ports, U., May, T., Blaha, T.,
894 Kreienbrock, L., 2007. The serological *Salmonella* monitoring in German pork
895 production: the structure of the central database and preliminary results of a basic
896 epidemiological report. Proceedings of the 7th International Safepork Symposium on
897 the epidemiology & control of foodborne pathogens in pork, may 9-11, Verona, Italy,
898 pages 140-144.
- 899 102. Methner, U., Rabsch, W., Reissbrodt, R., Williams, P.H., 2007. Effect of
900 norepinephrine on colonisation and systemic spread of *Salmonella enterica* in infected
901 animals: Role of catechol siderophore precursors and degradation products. Int. J.
902 Med. Microbiol. In Press.
- 903 103. Miao, E.A., Scherer, C.A., Tsois, R.M., Kingsley, R.A., Adams, L.G.,
904 Bauml, A.J., Miller, S.I., 1999. *Salmonella* Typhimurium leucine-rich repeat
905 proteins are targeted to the SPI1 and SPI2 type III secretion systems. Mol. Microbiol.
906 34, 850-864.
- 907 104. Miller, G.Y., Liu, X., McNamara, P.E., Barber, D.A., 2005. Influence of
908 *Salmonella* in pigs preharvest and during pork processing on human health costs and
909 risks from pork. J. Food. Prot. 68, 1788-1798.
- 910 105. Mikkelsen, L.L., Naughton, P.J., Hedemann, M.S., Jensen, B.B., 2004. Effects
911 of physical properties of feed on microbial ecology and survival of *Salmonella*
912 *enterica* serovar Typhimurium in the pig gastrointestinal tract. Appl. Environ.
913 Microbiol. 70, 3485-3492.
- 914 106. Mitchell, E.K., Mastroeni, P., Kelly, A.P., Trowsdale, J., 2004. Inhibition of
915 cell surface MHC class II expression by *Salmonella*. Eur. J. Immunol. 34, 2559-2567.
- 916 107. Molbak, K., Baggesen, D.L., Aarestrup, F.M., Ebbesen, J.M., Engberg, J.,
917 Frydendahl, K., Gerner-Smidt, P., Petersen, A.M., Wegener, H.C., 1999. An outbreak

- 918 of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype Typhimurium
919 DT104. N. Engl. J. Med. 341, 1420-1425.
- 920 108. Monack, D.M., Bouley, D.M., Falkow, S., 2004. *Salmonella* Typhimurium
921 persists within macrophages in the mesenteric lymph nodes of chronically infected
922 Nramp1^{+/+} mice and can be reactivated by IFN γ neutralization. J. Exp. Med.
923 199, 231-241.
- 924 109. Mossel, D.A.A., Morris, G.P., Struijk, C.B., Cowden, J.M., Browning, L.M.,
925 2003. Providing an adequate supply of microbiologically safe and palatable food and
926 drinking water: contribution of a European vertically integrated approach to educating
927 professionals and consumers-part I. Food protection trends. 23, 14-23.
- 928 110. Mossong, J., Even, J., Huberty-Krau, P., Schneider, F., 2006. Substantial
929 reduction of human *Salmonella* Enteritidis infections in Luxembourg in 2005.
930 Eurosurveillance 2006. 11, issue 1
931 <http://www.eurosurveillance.org/ew/2006/060119.asp#0>
- 932 111. Mrsny, R.J., Gewirtz, A.T., Siccardi, D., Savidge, T., Hurley, B.P., Madara,
933 J.L., McCormick, B.A., 2004. Identification of hepxilin A3 in inflammatory events: a
934 required role in neutrophil migration across intestinal epithelia. Proc. Natl. Acad. Sci.
935 U.S.A. 101, 7421-7426.
- 936 112. Nielsen, B., Alban, L., Stege, H., Sorensen, L.L., Mogelmoose, V., Bagger, J.,
937 Dahl, J., Baggesen, D.L., 2001. A new *Salmonella* surveillance and control
938 programme in Danish pig herds and slaughterhouses. Berl. Munch. Tierarztl.
939 Wochenschr. 114, 323-326.
- 940 113. Niewold, T.A., Veldhuizen, E.J., van der Meulen, J., Haagsman, H.P., de Wit,
941 A.A., Smits, M.A., Tersteeg, M.H., Hulst, M.M., 2007. The early transcriptional

- 942 response of pig small intestinal mucosa to invasion by *Salmonella enterica* serovar
943 Typhimurium DT104. Mol. Immunol. 44, 1316-1322.
- 944 114. Nishio, M., Okada, N., Miki, T., Haneda, T., Danbara, H., 2005. Identification
945 of the outer-membrane protein PagC required for the serum resistance phenotype in
946 *Salmonella enterica* serovar Choleraesuis. Microbiology. 151, 863-873.
- 947 115. Nollet, N., Houf, K., Dewulf, J., Catry, B., De Zutter, L., De Kruif, A., Maes,
948 D., 2006. Variability in antimicrobial resistance among *Salmonella enterica* strains
949 from fattening pigs and sows. Microb. Drug Resist. 12, 74-81.
- 950 116. Nollet, N., Maes, D., Duchateau, L., Hautekiet, V., Houf, K., Van Hoof, J., De
951 Zutter, L., De Kruif, A., Geers, R., 2005. Discrepancies between the isolation of
952 *Salmonella* from mesenteric lymph nodes and the results of serological screening in
953 slaughter pigs. Vet. Res. 36, 545-555.
- 954 117. Norris, F.A., Wilson, M.P., Wallis, T.S., Galyov, E.E., Majerus, P.W., 1998.
955 SopB, a protein required for virulence of *Salmonella* Dublin, is an inositol phosphate
956 phosphatase. Proc. Natl. Acad. Sci. U.S.A. 95, 14057-14059.
- 957 118. Oliveira, C.J., Carvalho, L.F., Garcia, T.B., 2006. Experimental airborne
958 transmission of *Salmonella* Agona and *Salmonella* Typhimurium in weaned pigs.
959 Epidemiol. Infect. 134, 199-209.
- 960 119. Oliveira, C.J., Garcia, T.B., Carvalho, L.F., Givisiez, P.E., 2007. Nose-to-nose
961 transmission of *Salmonella* Typhimurium between weaned pigs. Vet Microbiol. 125,
962 355-361
- 963 120. Osterberg, J., Vagsholm, I., Boqvist, S., Lewerin, S.S., 2006. Feed-borne
964 outbreak of *Salmonella* Cubana in Swedish pig farms: risk factors and factors
965 affecting the restriction period in infected farms. Acta Vet. Scand. 47, 13-21.

- 966 121. Padungtod, P., Kaneene, J.B., 2006. *Salmonella* in food animals and humans in
967 northern Thailand. *Int. J. Food Microbiol.* 108, 346-354.
- 968 122. Pampusch, M.S., Bennaars, A.M., Harsch, S., Murtaugh, M.P., 1998. Inducible
969 nitric oxide synthase expression in porcine immune cells. *Vet. Immunol.*
970 *Immunopathol.* 61, 279-289.
- 971 123. Paulin, S.M., Jagannathan, A., Campbell, J., Wallis, T.S., Stevens, M.P., 2007.
972 Net replication of *Salmonella enterica* serovars Typhimurium and Choleraesuis in
973 porcine intestinal mucosa and nodes is associated with their differential virulence.
974 *Infect. Immun.* 75, 3950-3960.
- 975 124. Phan, T.T., Khai, L.T., Ogasawara, N., Tam, N.T., Okatani, A.T., Akiba, M.,
976 Hayashidani, H., 2005. Contamination of *Salmonella* in retail meats and shrimps in the
977 Mekong Delta, Vietnam. *J. Food Prot.* 68, 1077-1080.
- 978 125. Poppe, C., Ziebell, K., Martin, L., Allen, K., 2002. Diversity in antimicrobial
979 resistance and other characteristics among *Salmonella* Typhimurium DT104 isolates.
980 *Microb. Drug Resist.* 8, 107-122.
- 981 126. Prouty, A.M., Gunn, J.S., 2000. *Salmonella enterica* serovar Typhimurium
982 invasion is repressed in the presence of bile. *Infect. Immun.* 68, 6763-6769.
- 983 127. Proux, K., Cariolet, R., Fravalo, P., Houdayer, C., Keranflech, A., Madec, F.,
984 2001. Contamination of pigs by nose-to-nose contact or airborne transmission of
985 *Salmonella* Typhimurium. *Vet. Res.* 32, 591-600.
- 986 128. Qimron, U., Madar, N., Mittrucker, H.W., Zilka, A., Yosef, I., Blushtain, N.,
987 Kaufmann, S.H., Rosenshine, I., Apte, R.N., Porgador, A., 2004. Identification of
988 *Salmonella* Typhimurium genes responsible for interference with peptide presentation
989 on MHC class I molecules: Deltayej *Salmonella* mutants induce superior CD8+ T-cell
990 responses. *Cell. Microbiol.* 6, 1057-1070.

- 991 129. Rahman, H., Reissbrodt, R., Tschape, H., 2000. Effect of norepinephrine on
992 growth of *Salmonella* and its enterotoxin production. Indian. J. Exp. Biol. 38, 285-
993 286.
- 994 130. Rajic, A., Waddell, L.A., Sargeant, J.M., Read, S., Farber, J., Firth, M.J.,
995 Chambers, A., 2007. An overview of microbial food safety programs in beef, pork,
996 and poultry from farm to processing in Canada. J. Food Prot. 70, 1286-1294.
- 997 131. Rasmussen, M.A., Carlson, S.A., Franklin, S.K., McCuddin, Z.P., Wu, M.T.,
998 Sharma, V.K., 2005. Exposure to rumen protozoa leads to enhancement of
999 pathogenicity of and invasion by multiple-antibiotic-resistant *Salmonella enterica*
1000 bearing SG11. Infect. Immun. 73, 4668-4675.
- 1001 132. Reed, W.M., Olander, H.J., Thacker, H.L., 1986. Studies on the pathogenesis
1002 of *Salmonella* Typhimurium and *Salmonella* Choleraesuis var kunzendorf infection in
1003 weanling pigs. Am. J. Vet. Res. 47, 75-83.
- 1004 133. Riber, U., Lind, P., 1999. Interaction between *Salmonella* Typhimurium and
1005 phagocytic cells in pigs. Phagocytosis, oxidative burst and killing in
1006 polymorphonuclear leukocytes and monocytes. Vet. Immunol. Immunopathol. 67,
1007 259-270.
- 1008 134. Riber, U., Nielsen, J., Lind, P., 2004. In utero infection with PRRS virus
1009 modulates cellular functions of blood monocytes and alveolar lung macrophages in
1010 piglets. Vet. Immunol. Immunopathol. 99, 169-177.
- 1011 135. Roth, J.A. 1999. The immune system. In: Straw, B.E., D'Allaire, S.,
1012 Mengeling, W.L., Taylor, D.J. (Eds.), Diseases of Swine, 8th ed. Iowa State
1013 University Press, Iowa, pp. 799–820.
- 1014 136. Rostagno, M.H., Hurd, H.S., McKean, J.D., 2007. *Salmonella enterica*
1015 prevalence and serotype distribution in swine at slaughter. Proceedings of the 7th

- 1016 International Safepork Symposium on the epidemiology & control of foodborne
1017 pathogens in pork, may 9-11, Verona, Italy, pages 153-155.
- 1018 137. Roy, M.F., Malo, D., 2002. Genetic regulation of host responses to *Salmonella*
1019 infection in mice. *Genes Immun.* 3, 381-393.
- 1020 138. Salmon, D.E., Smith, T., 1886. The bacterium of swine plague. *Am. Mon.*
1021 *Microbiol. J.* 7, 204.
- 1022 139. Scharek, L., Tedin, K., 2007. The porcine immune system--differences
1023 compared to man and mouse and possible consequences for infections by *Salmonella*
1024 serovars. *Berl. Munch. Tierarztl. Wochenschr.* 120, 347-354.
- 1025 140. Schauser, K., Olsen, J.E., Larsson, L.I., 2004. Immunocytochemical studies of
1026 *Salmonella* Typhimurium invasion of porcine jejunal epithelial cells. *J. Med.*
1027 *Microbiol.* 53, 691-695.
- 1028 141. Schauser, K., Olsen, J.E., Larsson, L.I., 2005. *Salmonella* Typhimurium
1029 infection in the porcine intestine: evidence for caspase-3-dependent and -independent
1030 programmed cell death. *Histochem. Cell. Biol.* 123, 43-50.
- 1031 142. Segales, J., Domingo, M., Chianini, F., Majo, N., Dominguez, J., Darwich, L.,
1032 Mateu, E., 2004. Immunosuppression in postweaning multisystemic wasting syndrome
1033 affected pigs. *Vet. Microbiol.* 98, 151-158.
- 1034 143. Selander, R.K., Beltran, P., Smith, N.H., Helmuth, R., Rubin, F.A., Kopecko,
1035 D.J., Ferris, K., Tall, B.D., Cravioto, A., Musser, J.M., 1990. Evolutionary genetic
1036 relationships of clones of *Salmonella* serovars that cause human typhoid and other
1037 enteric fevers. *Infect. Immun.* 58, 2262-2275.
- 1038 144. Selke, M., Meens, J., Springer, S., Frank, R., Gerlach, G.F., 2007.
1039 Immunization of pigs to prevent disease in humans: construction and protective

- 1040 efficacy of a *Salmonella enterica* serovar Typhimurium live negative-marker vaccine.
1041 Infect. Immun. 75, 2476-2483.
- 1042 145. Sheppard, M., Webb, C., Heath, F., Mallows, V., Emilianus, R., Maskell, D.,
1043 Mastroeni, P., 2003. Dynamics of bacterial growth and distribution within the liver
1044 during *Salmonella* infection. Cell. Microbiol. 5, 593-600.
- 1045 146. Shi, J., Zhang, G., Wu, H., Ross, C., Blecha, F., Ganz, T., 1999. Porcine
1046 epithelial beta-defensin 1 is expressed in the dorsal tongue at antimicrobial
1047 concentrations. Infect. Immun. 67, 3121-3127.
- 1048 147. Skjolaas, K.A., Burkey, T.E., Dritz, S.S., Minton, J.E., 2006. Effects of
1049 *Salmonella enterica* serovars Typhimurium (ST) and Choleraesuis (SC) on chemokine
1050 and cytokine expression in swine ileum and jejunal epithelial cells. Vet. Immunol.
1051 Immunopathol. 111, 199-209.
- 1052 148. Smith, J. L., 2003. The role of gastric acid in preventing foodborne disease and
1053 how bacteria overcome acid conditions. J. Food Prot. 66, 1292-1303.
- 1054 149. Splichal, I., Trebichavsky, I., Muneta, Y., Mori, Y., 2002. Early cytokine
1055 response of gnotobiotic piglets to *Salmonella enterica* serotype Typhimurium. Vet.
1056 Res. 33, 291-297.
- 1057 150. Stabel, T.J., Fedorka-Cray, P.J., Gray, J.T., 2002. Neutrophil phagocytosis
1058 following inoculation of *Salmonella* Choleraesuis into swine. Vet. Res. Commun. 26,
1059 103-109.
- 1060 151. Steenhard, N.R., Jensen, T.K., Baggesen, D.L., Roepstorff, A., Moller, K.,
1061 2002. Excretion in feces and mucosal persistence of *Salmonella* ser. Typhimurium in
1062 pigs subclinically infected with *Oesophagostomum* spp. Am. J. Vet. Res. 63, 130-136.
- 1063 152. Steenhard, N.R., Roepstorff, A., Baggesen, D.L., Boes, J., Jensen, T.K.,
1064 Aasted, B., Ornbjerg, N., 2006. Studies on the interaction between *Salmonella*

- 1065 *enterica* ser. Typhimurium and intestinal helminths in pigs. Vet. Parasitol. 139, 158-
1066 167.
- 1067 153. Steinbach, G., Hartung, M., 1999. Attempt to estimate the share of human
1068 *Salmonella* infections, which are attributable to *Salmonella* originating from swine.
1069 Berl. Münch. Tierärztl. Wochenschr. 112, 296-300.
- 1070 154. Stoycheva, M.V., Murdjeva, M.A., 2006. Antimicrobial therapy of
1071 salmonellosis--current state and perspectives. Folia Med (Plovdiv). 48, 5-10.
- 1072 155. Su, L.H., Chiu, C.H., Chu, C., Ou, J.T., 2004. Antimicrobial resistance in
1073 nontyphoid *Salmonella* serotypes: a global challenge. Clin. Infect. Dis. 39, 546-551.
- 1074 156. Sun, H.S., Wang, L., Rothschild, M.F., Tuggle, C.K., 1998. Mapping of the
1075 natural resistance-associated macrophage protein1 (NRAMP1) gene to pig
1076 chromosome 15. Anim. Genet. 29, 138-140.
- 1077 157. Talavera-Rojas, M., Vazquez-Chagoyan, J.C., Flores-Bello, R., Robles-
1078 Gonzalez, F., Lagunas-Bernabe, S., Alonso-Fresan, M.U., 2007. GyrA gene mutations
1079 and fluoroquinolone resistance in *Salmonella* isolates from pigs in central Mexico.
1080 Vet. Rec. 160, 630-632.
- 1081 158. Thakur, S., Tadesse, D.A., Morrow, M., Gebreyes, W.A., 2007. Occurrence of
1082 multidrug resistant *Salmonella* in antimicrobial-free (ABF) swine production systems.
1083 Vet. Microbiol. [Epub ahead of print]
- 1084 159. Threlfall, E.J. 2000. Epidemic *Salmonella* Typhimurium DT 104-a truly
1085 international multiresistant clone. J. Antimicrob. Chemother. 46, 7-10.
- 1086 160. Tierrez, A., Garcia-del Portillo, F., 2005. New concepts in *Salmonella*
1087 virulence: the importance of reducing the intracellular growth rate in the host. Cell.
1088 Microbiol. 7, 901-909.

- 1089 161. Timoney, J.F., Gillespie, J.H., Scott, F.W., Barlough, J.E., 1988. Hagan and
1090 Bruner's microbiology and infectious diseases of domestic animals. Comstock
1091 Publishing Associates, Ithaca, N.Y.
- 1092 162. Tschäpe, H., Bockemühl, J., 2002. Lebensmittelübertragene salmonellose in
1093 Deutschland. Bundesgesundheitsbl. Gesundheitsforsch. Gesundheitsschutz. 45, 491-
1094 496.
- 1095 163. Tükel, C., Raffatellu, M., Chessa, D., Wilson, R.P., Akcelik, M., Baumler,
1096 A.J., 2006. Neutrophil influx during non-typhoidal salmonellosis: who is in the
1097 driver's seat? FEMS Immunol. Med. Microbiol. 46, 320-329.
- 1098 164. Uthe J.J., Royae, A., Lunney, J.K., Stabel, T.J., Zhao, S.H., Tuggle, C.K.,
1099 Bearson, S.M., 2007. Porcine differential gene expression in response to *Salmonella*
1100 *enterica* serovars Choleraesuis and Typhimurium. Mol. Immunol. 44, 2900-2914.
- 1101 165. Uzzau S., Brown, D.J., Wallis, T., Rubino, S., Leori, G., Bernard, S.,
1102 Casadesus, J., Platt, D.J., Olsen, J.E., 2000. Host adapted serotypes of *Salmonella*
1103 *enterica*. Epidemiol. Infect. 125, 229-255.
- 1104 166. Valdezate S., Vidal, A., Herrera-Leon, S., Pozo, J., Rubio, P., Usera, M.A.,
1105 Carvajal, A., Echeita, M.A., 2005. *Salmonella* Derby clonal spread from pork. Emerg.
1106 Infect. Dis. 11, 694-698.
- 1107 167. Van der Velden, A.W., Copass, M.K., Starnbach, M.N., 2005. *Salmonella*
1108 inhibit T cell proliferation by a direct, contact-dependent immunosuppressive effect.
1109 Proc. Natl. Acad. Sci. U.S.A. 102, 17769-17774.
- 1110 168. van der Wolf, P.J., van Schie, F.W., Elbers, A.R., Engel, B., van der Heijden,
1111 H.M., Hunneman, W.A., Tielen, M.J., 2001. Administration of acidified drinking
1112 water to finishing pigs in order to prevent *Salmonella* infections. Vet. Q. 23, 121-125.

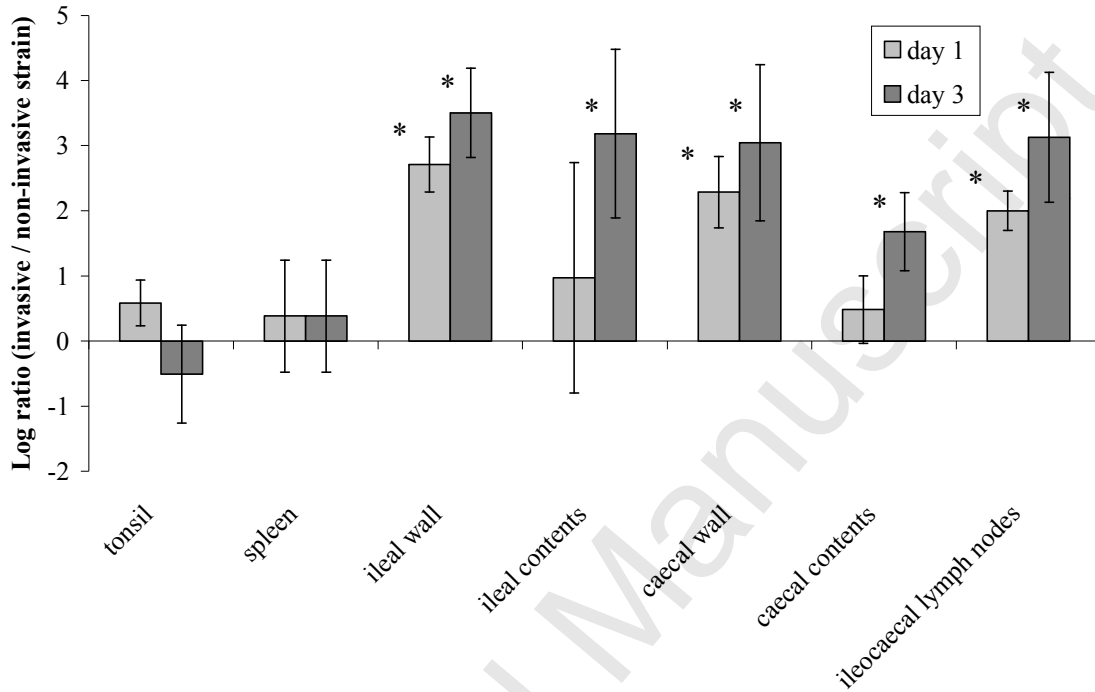
- 1113 169. Van Diemen, P.M., Kreukniet, M.B., Galina, L., Bumstead, N., Wallis, T.S.,
1114 2002. Characterisation of a resource population of pigs screened for resistance to
1115 salmonellosis. *Vet. Immunol. Immunopathol.* 88, 183-196.
- 1116 170. Van Immerseel, F., De Buck, J., Boyen, F., Bohez, L., Pasmans, F., Volf, J.,
1117 Sevcik, M., Rychlik, I., Haesebrouck, F., Ducatelle, R., 2004. Medium-chain fatty
1118 acids decrease colonization and invasion through *hilA* suppression shortly after
1119 infection of chickens with *Salmonella enterica* serovar Enteritidis. *Appl. Environ.*
1120 *Microbiol.* 70, 3582-3587.
- 1121 171. Van Immerseel, F., De Buck, J., De Smet, I., Pasmans, F., Haesebrouck, F.,
1122 Ducatelle, R., 2004. Interactions of butyric acid- and acetic acid-treated *Salmonella*
1123 with chicken primary cecal epithelial cells in vitro. *Avian Dis.* 48, 384-391.
- 1124 172. Van Immerseel, F., Russell, J.B., Flythe, M.D., Gantois, I., Timbermont, L.,
1125 Pasmans, F., Haesebrouck, F., Ducatelle, R., 2006. The use of organic acids to combat
1126 *Salmonella* in poultry: a mechanistic explanation of the efficacy. *Avian Pathol.* 35,
1127 182-188.
- 1128 173. Van Pelt, W., Van Giessen, A., Van Leeuwen, W., Wannet, W., Henken, A.,
1129 Evers, E., De Wit, M., Van Duynhoven, Y., 2000. Oorsprong, omvang en kosten van
1130 humane salmonellose. *Infectieziekten Bulletin.* 11, 4-8.
- 1131 174. van Velkinburgh, J.C., Gunn, J.S., 1999. PhoP–PhoQ-regulated loci are
1132 required for enhanced bile resistance in *Salmonella* spp. *Infect. Immun.* 67, 1614-
1133 1622.
- 1134 175. Velandar, H.I., Boes, J., Nielsen, B.J., Nielsen, B., 2007. Breeding for genetic
1135 resistance to *Salmonella* in pigs. Proceedings of the 7th International Safepork
1136 Symposium on the epidemiology & control of foodborne pathogens in pork, may 9-11,
1137 Verona, Italy, p 248-252.

- 1138 176. Veldhuizen, E.J., van Dijk, A., Tersteeg, M.H., Kalkhove, S.I., van der
1139 Meulen, J., Niewold, T.A., Haagsman, H.P., 2007. Expression of beta-defensins pBD-
1140 1 and pBD-2 along the small intestinal tract of the pig: Lack of upregulation in vivo
1141 upon *Salmonella* Typhimurium infection. *Mol. Immunol.* 44, 276-283.
- 1142 177. Vo, A.T., van Duijkeren, E., Fluit, A.C., Wannet, W.J., Verbruggen, A.J.,
1143 Maas, H.M., Gaastra, W., 2006. Antibiotic resistance, integrons and *Salmonella*
1144 genomic island 1 among non-typhoidal *Salmonella* serovars in The Netherlands. *Int. J.*
1145 *Antimicrob. Agents.* 28, 172-179.
- 1146 178. Volf, J., Boyen, F., Faldyna, M., Pavlova, B., Navratilova, J., Rychlik, I., 2007.
1147 Cytokine Response of Porcine Cell Lines to *Salmonella enterica* serovar
1148 Typhimurium and its *hilA* and *ssrA* mutants. *Zoonoses Public Health.* 54, 286-93.
- 1149 179. Wahlström, H., Eriksson, E., Noll, B., Plym Forsell, L., Wierup, M., Wollin,
1150 R., 2000. The Swedish control of pig and pork production during 1999. 16th IPVS
1151 Congres, September 17-21, Melbourne, Australia, p. 215.
- 1152 180. Wall, D.M., Nadeau, W.J., Pazos, M.A., Shi, H.N., Galyov, E.E., McCormick,
1153 B.A., 2007. Identification of the *Salmonella enterica* serotype Typhimurium SipA
1154 domain responsible for inducing neutrophil recruitment across the intestinal
1155 epithelium. *Cell. Microbiol.* 9, 2299-2313.
- 1156 181. Wang, Y.C., Yeh, K.S., Chang, C.C., Hsuan, S.L., Chen, T.H., 2006.
1157 Fluoroquinolone-resistant *Salmonella* sp. in carcasses. *Emerg. Infect. Dis.* 12, 351-
1158 352.
- 1159 182. Wang, Y., Qu, L., Uthe, J.J., Bearson, S.M., Kuhar, D., Lunney, J.K., Couture,
1160 O.P., Nettleton, D., Dekkers, J.C., Tuggle, C.K., 2007. Global transcriptional response
1161 of porcine mesenteric lymph nodes to *Salmonella enterica* serovar
1162 Typhimurium. *Genomics.* 90, 72-84.

- 1163 183. Waterman, S.R., Holden, D.W., 2003. Functions and effectors of the
1164 *Salmonella* pathogenicity island 2 type III secretion system. *Cell. Microbiol.* 5, 501-
1165 511.
- 1166 184. Wigley, P. 2004. Genetic resistance to *Salmonella* infection in domestic
1167 animals. *Res. Vet. Sci.* 76, 165-169.
- 1168 185. Wilcock, B. P., Schwartz, K., 1992. Salmonellosis. In: Leman, A.D., Straw,
1169 B.E., Mengeling, W.E., D'Allaire, S., Taylor, D.J. (Eds), *Diseases of Swine*, Iowa
1170 State University Press, Iowa, 570-583.
- 1171 186. Williams, P.H., Rabsch, W., Methner, U., Voigt, W., Tschape, H., Reissbrodt,
1172 R., 2006. Catecholate receptor proteins in *Salmonella enterica*: role in virulence and
1173 implications for vaccine development. *Vaccine.* 24, 3840-3844.
- 1174 187. Wills, R.W., Gray, J.T., Fedorka-Cray, P.J., Yoon, K.J., Ladely, S.,
1175 Zimmerman, J.J., 2000. Synergism between porcine reproductive and respiratory
1176 syndrome virus (PRRSV) and *Salmonella* Choleraesuis in swine. *Vet. Microbiol.* 71,
1177 177-192.
- 1178 188. Wood, R.L., Pospischil, A., Rose, R., 1989. Distribution of persistent
1179 *Salmonella* Typhimurium infection in internal organs of swine. *Am. J. Vet. Res.* 50,
1180 1015-1021.
- 1181 189. Wood, R.L., Rose, R., Coe, N.E., Ferris, K.E., 1991. Experimental
1182 establishment of persistent infection in swine with a zoonotic strain of *Salmonella*
1183 Newport. *Am. J. Vet. Res.* 52, 813-819.
- 1184 190. Ye, Z., Petrof, E.O., Boone, D., Claud, E.C., Sun, J., 2007. *Salmonella* Effector
1185 AvrA Regulation of Colonic Epithelial Cell Inflammation by Deubiquitination. *Am. J.*
1186 *Pathol.* 171, 882-892

- 1187 191. Zaidi, M.B., McDermott, P.F., Fedorka-Cray, F., Leon, V., Canche, C., Hubert,
1188 S.K., Abbott, J., Leon, M., Zhao, S., Headrick, M., Tollefson, L., 2006. Nontyphoidal
1189 *Salmonella* from human clinical cases, asymptomatic children, and raw retail meats in
1190 Yucatan, Mexico. Clin. Infect. Dis. 42, 21-28.
- 1191 192. Zhang, G., Wu, H., Ross, C.R., Minton, J.E., Blecha, F., 2000. Cloning of
1192 porcine *Nramp1* and its induction by lipopolysaccharide, tumor necrosis factor alpha,
1193 and interleukin-1 β : role of CD14 and mitogen-activated protein kinases. Infect.
1194 Immun. 68, 1086-1093.
- 1195 193. Zhang, S., Santos, R.L., Tsolis, R.M., Stender, S., Hardt, W.D., Baumler, A.J.,
1196 Adams, L.G., 2002. The *Salmonella enterica* serotype Typhimurium effector proteins
1197 SipA, SopA, SopB, SopD, and SopE2 act in concert to induce diarrhea in calves.
1198 Infect. Immun. 70, 3843-3855.
- 1199 194. Zhao, C., Ge, B., De Villena, J., Sudler, R., Yeh, E., Zhao, S., White, D.G.,
1200 Wagner, D., Meng, J., 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and
1201 *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater
1202 Washington, D.C., area. Appl. Environ. Microbiol. 67, 5431-5436.
- 1203 195. Zhao, S.H., Kuhar, D., Lunney, J.K., Dawson, H., Guidry, C., Uthe, J.J.,
1204 Bearson, S.M., Recknor, J., Nettleton, D., Tuggle, C.K., 2006. Gene expression
1205 profiling in *Salmonella* Choleraesuis-infected porcine lung using a long
1206 oligonucleotide microarray. Mamm. Genome. 17, 777-789.
- 1207 196. Zhou, D., Chen, L.M., Hernandez, L., Shears, S.B., Galan, J.E., 2001. A
1208 *Salmonella* inositol polyphosphatase acts in conjunction with other bacterial effectors
1209 to promote host cell actin cytoskeleton rearrangements and bacterial internalization.
1210 Mol. Microbiol. 39, 248-259.
- 1211

Figure 1



Recovery of bacteria from various organs of piglets at days 1 and 3 after inoculation with an equal mixture of an invasive field strain of *Salmonella* Typhimurium and an isogenic non-invasive mutant strain ($\Delta sipB$). The log value of the ratio of the number of cfu/g sample of the invasive and non-invasive strain is given as the mean \pm standard deviation. An asterisk indicates that the output ratio was significantly different ($p < 0.05$) from that present in the inoculum (adapted from Boyen et al., 2006c).

Table 1. *Salmonella* isolated from pork in various countries^a

Country	Year	Sampling	Sample	All serotypes		<i>Salmonella</i> Typhimurium
				N	%Pos	%Pos
USA	1999	Post-harvest	raw meat	209	3.3	ND
Great Britain	1999	harvest	carcasses	2509	5.3	2.1
Vietnam	2000	Post-harvest	raw meat	136	69.9	ND
Mexico	2001	Post-harvest	raw meat	339	58.1	5.1
Belgium	2004	harvest	raw meat	374	12.3	4.0
Denmark	2004	harvest	carcasses	34038	0.8	0.3
Finland	2004	harvest	carcasses	6576	0	-
Germany	2004	harvest	raw meat	4744	0.5	0.3
Hungary	2004	harvest	raw meat	8257	1.3	0.4
Italy	2004	harvest	raw meat	256	4.3	1.2
Italy	2004	harvest	carcasses	1096	3.6	0.9
Latvia	2004	harvest	raw meat	185	1.1	-
Malta	2004	harvest	raw meat	400	32.8	-
Poland	2004	harvest	raw meat	895	0.2	-
Portugal	2004	harvest	raw meat	256	15.2	-
Spain	2004	harvest	raw meat	147	10.2	-

^a Data are from references Zhao et al., 2001; EFSA, 2005; Davies et al., 2004; Phan et al., 2005; Zaidi et al., 2006.

Table 2. Overview of *Salmonella* virulence genes with reported role in *Salmonella*

Typhimurium pathogenesis in pigs

Virulence gene	Role in pathogenesis	References
FimA	Adhesion to intestinal epithelial cells	Althouse et al., 2003
SafA	Attenuated in STM screening	Carnell et al., 2007
SPI-1	Invasion in intestinal epithelial cells and development of enteritis	Boyen et al., 2006c; Brumme et al., 2007; Carnell et al., 2007
SPI-2	Attenuated for causing disease and in STM screening	Brumme et al., 2007; Carnell et al., 2007
SugR	Attenuated in STM screening	Carnell et al., 2007
MisL	Attenuated in STM screening	Carnell et al., 2007
MgtC	Attenuated in STM screening	Carnell et al., 2007
PipC	Attenuated in STM screening	Carnell et al., 2007
Various plasmid-associated genes	Attenuated in STM screening	Carnell et al., 2007
Various phage-associated genes	Attenuated in STM screening	Carnell et al., 2007
Various envelope/surface structures	Attenuated in STM screening	Carnell et al., 2007
Various house keeping genes	Attenuated in STM screening	Carnell et al., 2007
Various hypothetical proteins	Attenuated in STM screening	Carnell et al., 2007