Influence of long-time transportation stress on re-activation of *Salmonella* Typhimurium DT104 in experimentally infected pigs

Einfluss von Transportstress auf die Reaktivierung von *Salmonella* Typhimurium DT104 in experimentell infizierten Schweinen

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Summary:
In this study a *Salmonella* Typhimurium infection model in swine was used in order to investigate the influence of pre-mortal stress induced by long time period transportation on the re-activation of *Salmonella* in experimentally infected pigs. *Salmonella* free pigs were exposed to a highly virulent strain of *Salmonella* Typhimurium DT104 by direct intragastrical administration. Clinical parameters were monitored and the shedding rate in faeces was qualitatively and quantitatively determined by standard bacteriological procedures for 21 days. The distribution of the challenge organism in 14 different internal organs of transported and nontransported animals was determined. All infected animals developed clinical signs of salmonellosis 12 to 24 hours post infection. About 88 to 100 % of the fecal samples were culture-positive up to post exposure day 6, and then varied from 71 to 92 % until slaughter, respectively. At necropsy *S. Typhimurium* was recovered most frequently from caecum and ileocolic lymph nodes (83 %), colon (79 %), palatine tonsils (71 %) and mandibular lymph nodes (62,5 %). A negative impact of transportation stress on the shedding rate and the general condition of the animals was observed.

Keywords: Infection model, pre-mortal stress, shedding, inner organs, *Salmonella* Typhimurium

Introduction

Infections with *Salmonella enterica* are one of the main causes of human gastroenteritis. *Salmonella enterica* represent the most important agent in outbreaks of food-borne diseases around the world (Lacey, 1993). As published by the Robert Koch Institute (RKI, 2000), 85,000 cases of human Salmonellosis were reported in
Germany in 1999. In 27.7% S. Typhimurium was isolated. In Germany, 20% of human cases were caused by Salmonella originating from pork (Steinbach and Kroell, 1999), indicating the importance of meat or meat products contaminated with Salmonella as a potential health hazard for consumers (Baggesen and Wegener, 1994). One major problem with regard to identification and control is the occurrence of subclinically infected, asymptomatic carriers of S. Typhimurium DT104. Animals that survive DT104 infections usually become clinically inapparent but are colonized persistently with these bacteria. Those pigs are a reservoir for the contamination of other animals and pork products. They represent also a potential source for human infection. Wood et al. (1988) showed that convalescent pigs that have been exposed to S. Typhimurium continued to shed low numbers of the bacteria in their feces up to 28 weeks. In this study we have used a Salmonella infection model to investigate the influence of transportation stress on the distribution patterns of Salmonella in inner organs and on possible reactivation or increase of shedding rates of DT104 in experimentally infected pigs.

Materials and methods

Pigs
Twenty-four male hybrid pigs at 30 - 35 kg body mass were used in the challenge experiment and sixteen animals served as an uninfected control group. All animals were obtained from a single herd without any history of clinical salmonellosis. The feces of the pigs were tested bacteriologically negative for Salmonella before transfer to the experimental pens. The pigs were also serologically negative for Salmonella when tested with an LPS-ELISA. All animals were fed with a complete commercial antibiotic-free feed and had water ad libitum. Feed was withdrawn from the animals 20 hours before infection.

Experimental design
For the oral infection procedures, a highly virulent, penta-resistant isolate of S. Typhimurium DT104 originally isolated from a diseased pig was used. The strain was cultured in Luria-Broth (LB) at 37 °C overnight. About 2 ml of the culture were inoculated in 100 ml fresh, pre-warmed LB and grown to an optical density at 600 nm (OD$_{600}$) of 0.7 (mid-exponential growth phase). Cells were washed once in phosphate buffered saline (PBS), and re-suspended in 50 ml of pre-warmed PBS. Twenty-four animals were infected with 1 x 10$^{11}$ cells by intragastric application using a nasal stomach tube. All animals were sedated prior to infection (1.0 - 2.0 mg/kg azaperon, i.m.). Sixteen animals served as an uninoculated control group. At day 21 after challenge 12 animals (Inf$^+$ / Trans$^+$) were transported for 8 hours in an animal transport vehicle and slaughtered immediately. The remaining 12 animals (Inf$^+$ / Trans$^-$) were slaughtered without transportation. In the control experiment
8 non-infected animals remained untransported (Inf⁻/Trans⁺) and 8 animals (Inf⁻/Trans⁺) were transported under the same condition as described for the infected group. An overview of the experimental design is given in Table 1. In all pigs the presence of S. Typhimurium was quantitatively and qualitatively determined in the following tissues: palatine tonsils, mandibular lymph nodes, lung and lung lymph node, liver, spleen, segments from jejunum, ileum, colon and caecum, jejunal lymph nodes, ileocolic lymph nodes, colic lymph nodes, a piece of muscles of the forearm and a sample of the rectal contents.

Monitoring of clinical response
After infection, pigs were examined for changes in the clinical parameters. Rectal temperature, anorexia, signs of diarrhea and demeanour were monitored twice a day within the first 7 days and then daily. Shedding of S. Typhimurium was determined daily for 7 days by quantitative and qualitative culture of fecal samples.

Bacterial examinations - recovery of Salmonella from feces
Shedding of Salmonella in feces was determined daily within the first 6 days post-infection and then in intervals of 3 days until slaughter. Fecal samples of 1 g were inoculated in 9 ml of buffered peptone-water and homogenized by vortexing. To remove crude particles the suspension was centrifuged at low speed (300 x g) for 2 min. Twenty-five μl of the bacterial suspension containing supernatant was used for direct quantitative determination of Salmonella. A series of 10 fold dilutions of the supernatant were made in microtiter plates, using PBS as dilution medium. Twenty-five μl of each dilution (ranging from 1:10 to 1:10⁶) were pipetted threefold on XLD agar plates and incubated at 37 °C for 24 hours.
For qualitative analysis of Salmonella in feces, the remaining homogenizate was incubated for 18 to 24 hours at 37 °C. An aliquot of 0.1 ml of this suspension was transferred in 10 ml of Rappaport-Vassiliadis enrichment medium and incubated at 42 °C for further 18 to 24 hours and then streaked on XLD and BPLS agar plates. Plates were incubated 24 hours at 37 °C. Colonies having typical appearance of Salmonella were tested with Salmonella antiserum group B using slide agglutination.

Recovery of DT104 from inner organs
Tissue specimens of 5 g were immersed in 95 % ethanol, flamed, separately minced, and filled in a sterile plastic bag. Buffered peptone-water (1:9) was added and each sample was treated in a Stomacher 400 (Seward, London, UK) for 1 min at high speed. The following detection of Salmonella by selective-enrichment was carried out as described above.
Results

Clinical responses
All animals developed severe clinical signs of salmonellosis. The progression of the clinical response with regard to changes in signs of diarrhea, general demeanour, and appetite is shown in Figure 1. Twenty four to 36 hours post infection all of the infected pigs had viscous to watery, yellow feces. The prevalence of diarrhea varied in the first 7 days post exposure between 100 and 67 % and finally decreased to 25 % (n=4 pigs) in the following days. An individual variation in the level and the duration of the diarrhea was observed. Depression in demeanour was observed in 16 pigs (67 %), anorexia in 17 pigs (71 %) up to day 6 post infection. Two pigs (8 %) showed intense vomiting 12 hours post infection. Eighteen pigs (75 %) had a body temperature of up to 41 °C within 24 to 48 hours. Temperatures returned to normal within 6 days after exposure (data not shown).

Shedding of S. Typhimurium in feces
Strain DT104 was not detected before exposure and in the non-infected control groups. The shedding rate was determined quantitatively and qualitatively (Figure 2).
Post-infection, S. Typhimurium was isolated from the feces of all 24 infected pigs 2 days after oral application. During the first 6 days 87 – 100 % of the animals excreted DT104 in their feces. In the following days the excretion decreased to 90 – 71 % and 9 pigs excreted DT104 intermittently. The level of excretion varied from 4 x 10² to 6 x 10⁶ cfu/g feces in the first 7 days after infection and then decreased under the detection limit (4 x 10² cfu/g) for quantitative determination. At slaughter, S. Typhimurium was isolated from the sample of rectal content of 11 of the transported and of 7 of the unstressed pigs. Salmonella was not found in fecal samples and organs from pigs of the uninfected control group.

General distribution in inner organs
The frequency of recovery of DT104 from inner organs of the transported and unstressed pigs is given in Figure 3. The challenge organism was recovered most frequently and most consistently from the caecum (87.5 %), and the ileocolic lymph node (83 %), followed by the colon (79.1 %). From palatine tonsils (70.8 %), mandibular lymph node (62.5 %), cranial segments of the intestinal tract, and other intestinal lymph nodes recovery of the organism was less frequent. In one pig S. Typhimurium was isolated from muscles.

Influence of long-period transportation on shedding and distribution patterns of DT104 in inner organs
In comparison with the untransported group, an increased shedding rate was observed when animals were transported (Figure 3). Shedding of Salmonella was
observed in 92 % of the transported and 58 % of the pigs of in the untransported group. In addition to the increased shedding, transported animals had increased diarrhea and developed a disturbed general demeanour. No significant influence of transportation on the distribution of *Salmonella* in organ samples could be detected.

**Discussion**

Our results indicate that the applied infection model is suitable to induce an acute salmonellosis in pigs of 30 – 40 kg body mass constantly followed by intermittent shedding of DT104 in convalescent pigs and by persistent colonization of inner organs. Strain DT104 was most frequently recovered from the caecum and the ileocolic lymph nodes and less frequently from the tonsils and mandibular lymph nodes. This finding is in contradiction to the findings made by Wood et al. (1989), who stated that the infection strain was mainly isolated from the tonsils and less frequently from the gastrointestinal tract and adjacent lymph nodes. This might be caused by the specific properties of the strain used for infection. However, the high prevalence in ileocolic lymph nodes and caecum is in excellent accordance with the finding of Wood et al. (1989) and stresses the fact that the caecum is a reliable source for the *Salmonella* isolation. Therefore, the caecum and ileocolic lymph nodes represent important organs for sampling in slaughtering pigs. One main aim of this study was to investigate whether long-time (8 h) transportation effects the distribution of DT104 in inner organs and whether salmonellosis could be re-activated in all carrier pigs resulting in increased shedding rates at slaughter. Except the jejunum (Figure 3), no particular influence of the transportation on the distribution of DT104 was observed. This finding is in accordance with the findings of Isaacson et al. (1999). However, the shedding rate was increased in transported pigs. This indicates that clinically inapparent salmonellosis can be re-activated on long-time transportation.

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References


Table 1
Experimental design for studying effects of transportation stress on *Salmonella* Typhimurium DT104 infection in pigs. Groups of pigs, infection dose and transportation.

<table>
<thead>
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<th>Group of pigs</th>
<th>Number of pigs</th>
<th>Infection dose (cfu/animal)</th>
<th>8-hours transportation</th>
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<td>$1 \times 10^{11}$</td>
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<td>(Inf$^+$ / Trans$^+$)</td>
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<td></td>
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<tr>
<td>Infected-non &amp; transported</td>
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<td>$1 \times 10^{11}$</td>
<td>No</td>
</tr>
<tr>
<td>(Inf$^+$ / Trans$^-$)</td>
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<td></td>
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</tr>
<tr>
<td>Non infected &amp; transported</td>
<td>8</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>(Inf$^-$ / Trans$^+$)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Non infected &amp; non-transported</td>
<td>8</td>
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<td>No</td>
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<tr>
<td>(Inf$^-$ / Trans$^-$)</td>
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Figure 1
Clinical response to experimental S. Typhimurium DT104 infection in pigs. A, diarrhea; B, general demeanour; C, anorexia.
Figure 2
Shedding of S. Typhimurium DT104 in feces of experimentally infected pigs. A, quantitative bacteriological results; B, qualitative bacteriological results.
Figure 3
Distribution patterns of *S. Typhimurium* in inner organs of experimentally infected pigs. To = palatine tonsils, MaLy = mandibular lymph node, Lu = lung, Le = liver, Mi = spleen, Je = jejunum, Il = ileum, Co = colon, Cae = caecum, JeLy = jejunal lymph node, IcLy = ileocolic lymph node, CoLy = colic lymph node, Mus = musculature.