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Maternal immunity induced by inactivated *S.* Typhimurium vaccine is less protective to *S.* Derby challenge than to *S.* Typhimurium challenge in suckling piglets

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ABSTRACT: *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Derby are the most common serovars of *Salmonella enterica* ssp. *enterica* found in pigs in Europe. We previously observed that suckling piglets of sows vaccinated with an *S.* Typhimurium-based inactivated vaccine are protected against homologous strain challenge. To develop this vaccine for commercial use, potential crossprotectivity of this vaccine to challenge with *S.* Derby was tested. Two sows were vaccinated with an *S.* Typhimurium-based inactivated vaccine while two other sows remained serologically negative. Four-day-old suckling piglets from both groups were orally challenged with *S.* Derby or *S.* Typhimurium. Maternally-derived immunity against *S.* Typhimurium protected piglets against *S.* Typhimurium challenge, when a significant ($P < 0.05$) decrease in *S.* Typhimurium count was found in ileocaecal and submandibular lymph node, tonsil, ileum and ileum content. On the other hand, after *S.* Derby challenge, significant ($P < 0.05$) decrease in *S.* Derby count was detected only in ileum content. Although both serovars belong to the same O:4 serogroup, other antigenic structures, for example the flagellin, are different. In a subsequent in-vitro experiment, we found that serum from vaccinated sows inhibited the motility of *S.* Typhimurium but not the motility of *S.* Derby. Our results indicate that protectivity of *S.* Typhimurium vaccine against *S.* Derby infection is limited.

Keywords: vaccination; crossprotectivity; antibody

Abbreviations

BHI = brain-heart infusion, **ELISA** = Enzyme-Linked Immuno Sorbent Assay, **LPS** = lipopolysaccharide, **SD** = *Salmonella enterica* serovar Derby, **STM** = *Salmonella enterica* serovar Typhimurium

Pigs are the second most important source of *Salmonella* infection for humans in Europe after poultry (Anonymous 2009). One way to decrease a risk of *Salmonella* transmission from pig meat and slaughter products to humans is to decrease the number of *Salmonella*-positive pigs at slaughter. Piglets can be infected soon after birth when the infected sow or its environment is the infection source (Proux et al. 2001; Boughton et al. 2007). The immunity of newborn piglets could be crucial for eliminating this source of infection. Previously, we demonstrated that vaccination of pregnant sows with experimental inactivated *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) vaccine

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induces colostral immunity responsible for the protection of suckling piglets when challenged with a homologous strain (Matiasovic et al. 2013). Because *Salmonella enterica* serovar Derby (*S.* Derby) and *S.* Typhimurium belong to the same O:4 group (Grimont and Weill 2007), the aim of the study was to test potential crossprotectivity of *S.* Typhimurium vaccine to *S.* Derby challenge.

MATERIAL AND METHODS

The animal care protocol and its use in this experiment were approved by the Branch Commission for Animal Welfare of the Ministry of Agriculture of the Czech Republic (MZE1358). Four sows negative for *Salmonella*- specific antibodies, tested with Salmotype pig screen ELISA Kit (Labor Diagnostik, Germany) were used in the experiment. Two sows were vaccinated intramuscularly into the neck with 1 ml of inactivated *S.* Typhimurium vaccine four and two weeks before parturition and developed antibody response to the vaccine. The in-house vaccine was prepared from *S.* Typhimurium DT104 incubated overnight in BHI medium to 1×10^9 CFU per ml, inactivated with 1% formaldehyde and mixed with ISA50V2 adjuvant (Seppic, France) (Matiasovic et al. 2013). None of the sows shed *Salmonella* sp. one week before and four days after parturition when checked daily by the ISO 6579: 2002 bacterial culture method.

IgG antibodies were measured using Salmotype pig screen ELISA Kit and IgA antibodies with a homemade ELISA based on *S.* Typhimurium O-antigens (Matiasovic et al. 2013). Briefly, Maxisorp plates (Nunc, Denmark) were coated with *S.* Typhimurium LPS (Sigma-Aldrich, USA). Serum samples were diluted 100 times and jejunal lavage two times in PBS and applied to coated plates. After incubation, the secondary antibody (Goat anti-pig IgA conjugate, Bethyl Laboratories, USA) conjugated with horseradish peroxidase was added. Subsequently, TMB substrate (Test-line, Czech Republic) was applied and the signal was measured at 450 nm on microplate reader Synergy H1 (Biotek, USA).

Four days after birth, suckling piglets of one vaccinated (n = 10) and one serologically negative sow (n = 8) were orally inoculated with 2.4×10^7 CFU of *S.* Typhimurium (STM challenge group) homologous to the vaccine strain (dose volume 2 ml). Similarly, piglets of two other sows (n = 10 in each group) were orally inoculated with 2.6×10^7 CFU of *S.* Derby (SD challenge group) (Matiasovic et al. 2014). All animal groups were individually housed in barrier pens. All piglets remained with their mothers until euthanized by exsanguination from the arteria brachialis under deep anaesthesia at 72 h post inoculation, i.e. at the end of the experiment when the *Salmonella* load within tissues was anticipated to be maximal (Wood and Rose 1992).

Quantitative bacteriology was performed by plating ten-fold serial dilutions of homogenised organ samples on XLD agar plates. After direct plating, counts of *Salmonella* were logarithmically transformed. Samples negative for *Salmonella* according to the aforementioned method, were subjected to the enrichment in semi-solid Rappaport-Vassiliadis medium (Oxoid, Basingstoke, United Kingdom) for qualitative *Salmonella* determination. Samples positive only after enrichment were taken as a value of one, and a value of zero was assigned to negative samples.

Influence of anti-*S.* Typhimurium antibodies on *S.* Typhimurium and *S.* Derby motility was tested *in vitro* according to Forbes et al. (2008). LB agar (0.3 g of agar per 100 ml, Invitrogen, USA) plates without antibodies or containing 100 × or 1000 × diluted heat-inactivated serum from both vaccinated sows were inoculated with 1 µl of overnight culture of *S.* Typhimurium or *S.* Derby. The diameter of migrating bacteria was measured in duplicates each hour of 8 h of cultivation. A test was performed four times, twice for serum from each sow. *Salmonella*-specific antibody levels in the serum samples of both sows were 86% and 116% of positive control of Salmotype pig screen ELISA Kit.

The significance of differences among groups for *in vivo* and for *in vitro* experiments was tested by ANOVA followed by the Bonferroni's Multiple Comparison Test. Differences with $P < 0.05$ were considered significant.

RESULTS

On Day Two after inoculation, piglets in all groups developed mild diarrhoea. The only clinical difference among groups was a slightly, but significantly higher body temperature at day two after inoculation in piglets from the serologically negative sow (39.8 °C ± 0.3), when compared to their counterparts from the vaccinated sow (39.3 °C ± 0.3) in *S.* Typhimurium challenge group.

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Table 1. *Salmonella*-specific antibody levels in the blood and jejunal lavage from suckling piglets

Challenge group	IgG DPI 0	IgG DPI 3	IgA DPI 0	IgA DPI 3	IgA DPI 3 jejunum
SD; Ab neg.; *n* = 10	–0.52 ± 0.37[a]	–1.08 ± 0.30[a]	0.20 ± 0.02[a]	0.12 ± 0.01[a]	0.31 ± 0.05[a]
STM; Ab neg.; *n* = 8	6.41 ± 2.18[a]	4.20 ± 1.52[a]	0.25 ± 0.03[a]	0.13 ± 0.01[a]	0.63 ± 0.15[ac]
SD; vacc.; *n* = 10	110.70 ± 2.43[b]	102.70 ± 3.38[b]	1.92 ± 0.09[b]	1.07 ± 0.04[b]	1.51 ± 0.10[b]
STM; vacc.; *n* = 10	100.10 ± 1.47[c]	95.89 ± 1.19[b]	2.02 ± 0.06[b]	1.14 ± 0.07[b]	0.76 ± 0.09[c]

DPI = day post infection, SD = *S.* Derby challenge, STM = *S.* Typhimurium challenge, Ab neg. = piglets from the sow negative for *Salmonella*-specific antibodies, vacc. = piglets from the vaccinated sow, *n* = number of animals in group
Values are presented as a mean ± SEM. Within a column, values with different superscripts ([a, b, c]) differ significantly ($P < 0.05$)

Piglets from both vaccinated sows had significantly higher amounts of *Salmonella*-specific IgG and IgA antibodies in the blood (Table 1) than piglets from serologically negative sows. Piglets from the vaccinated sow in the SD challenge group had slightly, but significantly higher amounts of IgG at Day 0 post infection (DPI 0) than piglets from the vaccinated sow in the STM challenge group. The levels of specific IgA in jejunal lavage in piglets of the vaccinated sow in the SD challenge group were significantly higher than in piglets from the serologically negative sow, whereas in the STM challenge group, the difference between piglets from vaccinated and serologically negative sows was not significant.

Piglets from the vaccinated sow in the STM challenge group had a significantly lower *Salmonella* Typhimurium count in the ileocaecal lymph node, submandibular lymph node, tonsil, ileum wall and ileum content than piglets from the serologically negative sow (Table 2). The spleen was not colonised in any animal and the liver was positive only after enrichment in two piglets from the serologically negative sow. This difference was not statistically significant.

Piglets from the vaccinated sow in the SD challenge group had a significantly lower *S.* Derby count in ileum content than piglets from the serologically negative sow (Table 2). However, *S.* Derby counts in the ileocaecal lymph node, submandibular lymph node, tonsil and ileum were not significantly different. The spleen and liver were *S.* Derby negative in all animals.

DISCUSSION

Although piglets from the vaccinated sow challenged with *S.* Derby acquired high levels of anti- *S.* Typhimurium antibodies (even higher than piglets from the vaccinated sow challenged with *S.* Typhimurium), their protection against *S.* Derby infection, measured as a significant decrease in *Salmonella* counts in tissues, was lower than in the *S.* Typhimurium challenge group. This might have been influenced by overall lower colonisation of organs by *S.* Derby, but also by differences in protein sequences between *S.* Derby and *S.* Typhimurium. Although both serovars have the same structure of O-antigens, the sequence of fliC protein, the major component of the flagellum, shows 78% similarity

Table 2. *Salmonella enterica* serovar Derby or Typhimurium counts in tissues

Challenge group	Spleen	Liver	IC LN	SM LN	Tonsil	Ileum wall	Ileum content
SD; Ab neg.; *n* = 10	0.00	0.00	1.24 ± 0.26	1.75 ± 0.42	0.30 ± 0.15	2.50 ± 0.46	2.49 ± 0.72
SD; vacc.; *n* = 10	0.00	0.00	0.30 ± 0.15	0.68 ± 0.39	0.10 ± 0.10	2.37 ± 0.56	1.00 ± 0.00*
STM; Ab neg.; *n* = 8	0.00	0.25 ± 0.16	4.71 ± 0.17	3.90 ± 0.29	2.51 ± 0.40	6.11 ± 0.28	4.94 ± 1.37
STM; vacc.; *n* = 10	0.00	0.00	1.91 ± 0.47*	1.08 ± 0.42*	0.81 ± 0.50*	4.02 ± 0.27*	2.50 ± 0.22*

SD = *S.* Derby challenge, STM = *S.* Typhimurium challenge, Ab neg. = piglets from the sow negative for *Salmonella*-specific antibodies, vacc. = piglets from the vaccinated sow, IC = ileocaecal, LN = lymph node, SM = submandibular, *n* = number of animals in group
Data for the sampled tissues are presented as a mean ± SEM of log 10 values of *S.* Typhimurium or *S.* Derby CFU/g for each group. Asterisks indicate the significance of differences ($P < 0.05$) between piglets from vaccinated and serologically negative sow within challenge group

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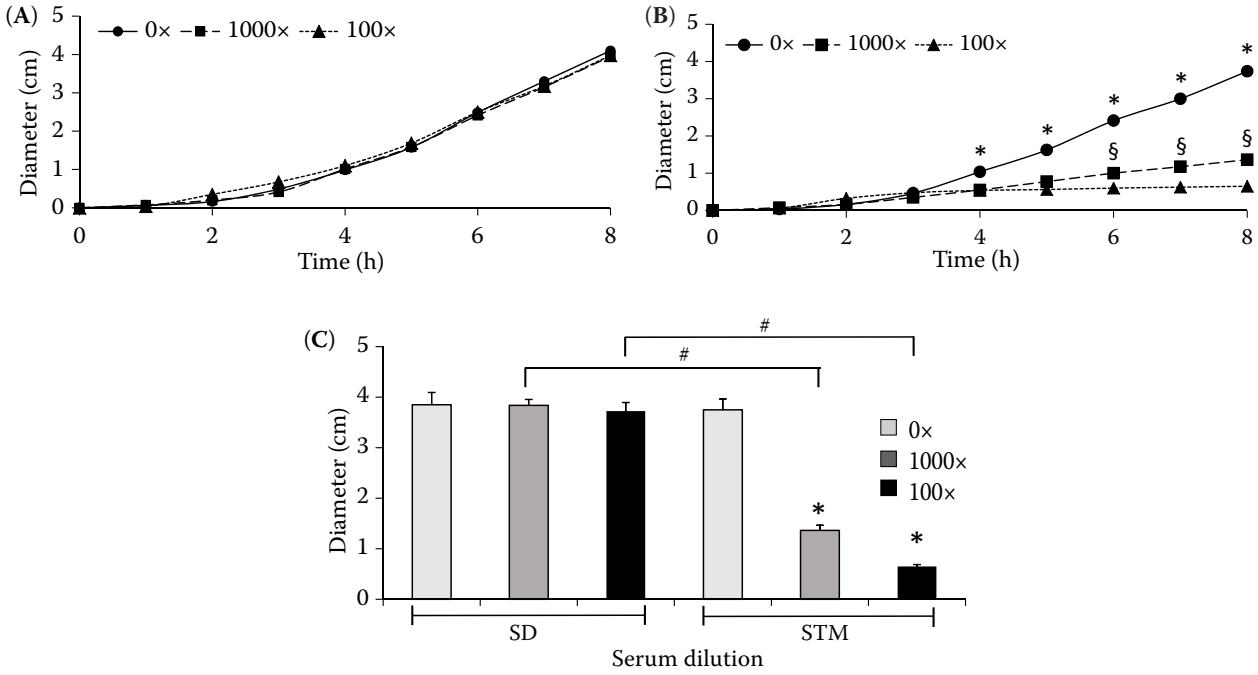

Figure 1. Influence of anti-*S.* Typhimurium antibodies on *S.* Typhimurium and *S.* Derby motility. The diameter of *S.* Derby (**A**) and *S.* Typhimurium (**B**) bacterial ring was gradually measured in agar plates with different concentrations of antibodies against *S.* Typhimurium (0 ×= without serum, 1000 × and 100 × diluted serum) incubated for 8 h. The last hour, motility of *S.* Derby and *S.* Typhimurium was compared (**C**). Each image represents the average of four separated experiments. *, # or § represents *P*-value of less than 0.05 that was considered significant. SD = *S.* Derby; STM = *S.* Typhimurium

* = inhibition of bacterial motility in 1000 × and 100 × diluted serum when compared to plates without serum

§ = difference in motility between 1000 × and 100 × dilution of serum

= significance between *S.* Derby and *S.* Typhimurium motility on the plates with the same serum dilutions

only to the first 190 amino acids (aa) from about 500 aa forming the whole protein (STM 495 aa, SD 504 aa) when compared by blastp using default settings (http://blast.ncbi.nlm.nih.gov). In accordance with this finding, the motility test showed that *in vitro* anti-*S.* Typhimurium antibodies significantly reduced the motility of *S.* Typhimurium but not *S.* Derby (Figure 1). In this test, the presence of anti-*S.* Typhimurium antibodies in LB semi-solid agar significantly reduced motility of *S.* Typhimurium from 4 h post inoculation. Under the same conditions the motility of *S.* Derby was not significantly reduced. The motility of both serovars on plates without antibodies was not significantly different. Another important antigenic target for protective antibodies is membrane protein ompD (Gil-Cruz et al. 2009), which has 89% similarity between these two serovars. Different epitopes of *S.* Derby proteins thus may limit the protectivity of antibodies induced by *S.* Typhimurium vaccine.

In the past, an effort was made to test crossprotectivity of *S.* Choleraesuis live attenuated vaccine to *S.* Typhimurium challenge. In a controlled study it was found that live attenuated *S.* Choleraesuis vaccine did not reduce shedding (Letellier et al. 2000), but could stimulate local immunity and reduce the presence of *S.* Typhimurium in the ileum in swine (Letellier et al. 2001). Some field studies found that live attenuated *S.* Choleraesuis vaccine reduced seroprevalence and *Salmonella* isolation in pigs at slaughter (Maes et al. 2001; Schwarz et al. 2011), whereas others, working with a vaccine based on *S.* Typhimurium var. Copenhagen, the crossprotectivity did not observed (Farzan and Friendship 2010).

It has to be taken into account that our data were obtained from a limited number of animals. Nevertheless, it was found that crossprotectivity of antibodies developed after vaccination was reduced between *Salmonella* Typhimurium and Derby serovars.

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