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Research paper

Protective immunity to *Salmonella enterica* is partially serogroup specific

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ABSTRACT

Pre-harvest reduction of *Salmonella* carriage by swine would benefit both animal health and food quality. While vaccination is an attractive pre-harvest intervention to reduce *Salmonella* levels in swine, the large number of potential *Salmonella enterica* serovars found in swine makes it critical that vaccines provide broad serotype efficacy. In order to directly compare the relative efficacy of *Salmonella* vaccines against serogroup-matched and serogroup-unmatched *Salmonella*, we vaccinated pigs with two commercially available *Salmonella* vaccines (either serogroup B or serogroup C1) and challenged with serovar-matched, serogroup-matched or serogroup-unmatched challenge strains. We found that while serogroup-matched vaccines provided relatively better efficacy than unmatched vaccines, serotype-unmatched vaccines also provided significant reduction of *Salmonella* carriage and shed. In addition, by measuring serogroup specific cell mediated (IFN-γ ELISPOT) and humoral (anti-LPS ELISA) immunity, we found that this serogroup specific efficacy correlates primarily with humoral immunity, while cell mediated immunity was mostly non-serogroup specific. While the practical relevance to pork quality of this serogroup-specific efficacy remains to be demonstrated, the large predominance of serogroup B *Salmonella* in swine suggests that a serogroup B *Salmonella* vaccine for swine would be of value to pre-harvest food safety interventions in swine.

1. Introduction

Salmonella enterica infections of domestic food production animals pose potential risk to animal health and meat quality. Implementation of post-harvest interventions programs has improved the bacteriological quality of pork over the last decade. Percent positive *Salmonella* tests in the Pathogen Reduction: Hazard Analysis and Critical Control Point (PR/HACCP) verifications program has decreased from the baseline of 8.7% in 1998 to a low of 2.3% in 2009, but then increased to 3.3% by 2011 (USDA, 2012). While post-harvest intervention has been of significant benefit, additional and sustained benefits to meat quality and animal health may be realized by pre-harvest reductions in *Salmonella* carriage, including by vaccination.

Salmonella vaccines are generally effective in reducing *Salmonella* prevalence at or near the time of slaughter (Denagamage et al., 2007). However, the use of

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vaccines to reduce *Salmonella* carriage is complicated by the array of different serovars found in swine. While the majority of clinical Salmonellosis in swine is attributed to *S. enterica* serovar Typhimurium (*Salmonella* Typhimurium; serogroup B) and *S. enterica* serovar Choleraesuis (*Salmonella* Choleraesuis; serotype C1), many other serovars are commonly reported in swine. In 2006 the top ten most common serovars isolated from pigs and typed at the National Veterinary Service Laboratory (NVSL) included serogroups B, C1, C2, E1 and E4, with 77.1% of these most common isolates being serotype B (USDA, 2007a). Worldwide, the majority of *S. enterica* serovars found in swine are serogroup B, including predominantly *S.* Typhimurium and *Salmonella* Derby (Boyen et al., 2008). From 1995 to 2006 the three most common serovars found in surveys of US pig operations were *S.* Derby, *S.* Typhimurium (var. 5-, formerly var. Copenhagen) and *Salmonella* Agona. These three serogroup B *Salmonella* accounted for 48% of all isolates in 2006 (USDA, 2007b). The role of swine as a source of human *Salmonella* infection is significant and has been previously reviewed (Boyen et al., 2008). For example, in European countries 15–23% of human cases are thought to originate from pork (reviewed in (Boyen et al., 2008)) and in the US a "farm-to-fork" model was used to estimate that about 100,000 cases of human Salmonellosis were associated with pork annually (Miller et al., 2005). Five of the ten most common *Salmonella* serovars isolated from pigs (2006) were also among the top 20 serovars isolated from humans in 2009 (CDC, 2011). Of the isolates comprising these five swine-derived serotypes, 95% are serotype B. Thus, strategies such as vaccination to reduce shedding and colonization of *Salmonella* in pork and in particular those which specifically address *S.* Typhimurium and other serotype B *Salmonella* could potentially have a positive impact on pork quality.

Current avirulent, live-culture swine *Salmonella* vaccines are based on *S.* Choleraesuis (serotype C1) (Anonymous, 2008). In addition to mitigating clinical Salmonellosis, these vaccines reduce shedding and carcass contamination, and provide some cross-protection to other serotypes of *Salmonella*, including *S.* Typhimurium (Braum, 1997; Husa et al., 2009). Cross-serogroup vaccine efficacy has also been noted in cattle (Mohler et al., 2008).

Since the ability of *Salmonella* vaccines to have a significant impact on food safety may depend on the degree of cross protection to varied *Salmonella* serogroups, we directly compared the impact of two modified-live *Salmonella* vaccines (serotypes B and C1) shedding and colonization of serotype matched and unmatched *Salmonella* challenge strains. In addition, we investigated the role of two immune effector mechanisms (anti-LPS antibodies and *Salmonella* specific IFN-γ secreting cells) in the observed serogroup-specificity of vaccine efficacy.

2. Materials and methods

2.1. Animal studies

All three animal studies were conducted in accordance with the Zoetis Institutional Animal Care and Use Committee (IACUC) guidelines and the IACUC guidelines of all contract research organizations involved. Actual study execution was divided by challenge strain for reasons of animal care and housing as well as biosecurity (preventing cross-contamination of different challenge strains). Specifics of the studies including number of animals, vaccines and challenges are listed in Table 1. In all studies, pigs were separated by treatment group for the vaccination phase of the study, but were comingled following challenge. Initial studies found that the vaccine strains did not shed beyond 3 days post vaccination, so the post-challenge comingling of pigs was not considered a risk for vaccine spread between groups. Studies 1 and 2 were conducted to assess the relative efficacy of serovar matched, serogroup matched and serogroup unmatched vaccines. In Study 1, pigs were vaccinated at 3 weeks of age with a live *S. enterica* serovar Typhimurium (*S.* Typhimurium) vaccine (MeganVac®1) as described below (ST_{vac}), challenged 3 weeks later with either a high or low (approximately 1×10^{10} or 1×10^{6} CFU, respectively) of a serovar matched (*S.* Typhimurium), serogroup matched (*S.* Derby) or serogroup unmatched (*S.* Infantis) *Salmonella* and were necropsied 4 weeks following challenge. Fecal shedding of *Salmonella* was monitored following challenge. At necropsy *Salmonella* were enumerated from cecal contents, terminal ileum (ileal–cecal junction) and mesenteric lymph nodes. In Study 2, pigs were vaccinated at 6 weeks of age with either MeganVac®1 (ST_{vac}) or Argus®SC/ST (SC_{vac}) as described below and were challenged 3 weeks later with either serovar-matched, serogroup matched or serogroup-unmatched challenges (*S.* Typhimurium or *S.* Derby) and were necropsied 4 weeks following challenge. Fecal shedding of *Salmonella* and tissue colonization was monitored as for Study 1. In a third study, sera from pigs that had been vaccinated (6 weeks of age) and challenged (3–4 weeks later) with either *S.* Typhimurium or *S.* Choleraesuis were used to assess the serotype specificity of the immune response. Blood was

Table 1
Study design, animal numbers and reference to Figures and Tables of results.

Study	Vaccine[a]	Challenge[a]	Pigs per group
1	ST_{vac}	$ST_{chal\text{-}low\ dose}$	12
		$ST_{chal\text{-}high\ dose}$	18
		$SD_{chal\text{-}low\ dose}$	10
		$SD_{chal\text{-}high\ dose}$	10
		$SI_{chal\text{-}low\ dose}$	10
		$SI_{chal\text{-}high\ dose}$	10
	Placebo	$ST_{chal\text{-}low\ dose}$	12
		$ST_{chal\text{-}high\ dose}$	18
		$SD_{chal\text{-}low\ dose}$	10
		$SD_{chal\text{-}high\ dose}$	10
		$SI_{chal\text{-}low\ dose}$	10
		$SI_{chal\text{-}high\ dose}$	10
2	ST_{vac}	ST_{chal}	20
		SD_{chal}	20
	SC_{vac}	ST_{chal}	20
		SD_{chal}	20
	Placebo	ST_{chal}	20
		SD_{chal}	20
3	ST_{vac}	ST_{chal}	15
	SC_{vac}	SC_{chal}	12

[a] See Table 2 for descriptions of vaccine and challenge strains and dosages.