

### 32. Single nucleotide polymorphisms (SNPs) in genes related to innate immune response against *Salmonella* in nursery pigs

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#### Abstract

The objective of this study was to investigate the association of single nucleotide polymorphisms (SNPs) in innate immune response genes with *Salmonella* shedding in nursery pigs. One hundred and sixty eight pigs on seven farrow-to- finish farms and one farrow-feeder operation were included in the study. On each farm, 21 pigs were selected from seven sows at weaning. Fecal samples were collected from selected pigs and cultured for *Salmonella*, and the isolates were serotyped. DNA was extracted from liver samples and used to genotype pigs for single nucleotide polymorphisms (SNPs) in 21 different innate immune response genes. In total, 15 (9.3%) pigs tested positive for *Salmonella*; the isolates from six pigs from four different litters on one farm were serotyped as *Salmonella* Infantis and from nine pigs from six different litters on another farm as *Salmonella* Worthington. SNP analysis showed an association of *Salmonella* shedding with a SNP in the genes encoding mannan-binding lectin (MBL)-associated serine protease-2 (MASP-2) and Toll-like receptor-1 (TLR-1) ( $P < 0.05$ ). These findings suggest that *Salmonella* shedding in pigs is controlled by genetic elements and these genetic variants could possibly be used to breed pigs that are more resistant to *Salmonella* colonization and *Salmonella* shedding.

#### Introduction

*Salmonella* is estimated to cause more than 1.2 million illnesses each year in the United States, with more than 23,000 hospitalizations (the leading cause of hospitalization due to foodborne illnesses), and 450 deaths (Scallan *et al.*, 2011). In Canada, it is estimated that there are 88,000 human cases of salmonellosis annually (Thomas *et al.*, 2011). Painter *et al.* (2013) have recently estimated that 46 % and 22 % of annual gastroenteritis cases associated with foodborne outbreaks in the United States are attributed to produce (fruits, nuts, and vegetable) and meat (beef, pork, and poultry) commodities, respectively. Asymptomatic pigs play a significant role in the spread and transmission of *Salmonella* from farm to fork. In fact, a major food safety concern is that *Salmonella* can be transmitted from pig farms to pork products at slaughterhouses, as well as to ground water and produce if the pig manure is used as land fertilizer. Despite a variety of suggested control measures for *Salmonella*, asymptomatic carrier pigs are very difficult to eliminate. As such, genetic selection for resistance against a *Salmonella* carrier-state could be utilized as an effective method to control *Salmonella* in pigs. However, the genetic markers causing the variation in response to *Salmonella* colonization and shedding, as part of the innate, cell-mediated and/or antibody mediated responses among pigs are still unknown. The objective of this study is to identify if single nucleotide polymorphisms in genes involved in pattern recognition and innate immunity are associated with altered *Salmonella* colonization and carrier-state status.

#### Material and Methods

Eight swine farms (7 farrow-to- finish farms and one farrow-feeder farm) in southern Ontario, Canada were included in this study. On each farm, 21 piglets were selected from 6-7 sows at weaning; in total 168 piglets (21 piglets per farm) were selected from 55 sows. Fecal samples were collected for *Salmonella* culturing at weaning. The piglets were euthanized at 5 weeks post-weaning and tissue samples collected from

liver for DNA extraction. DNA was extracted from liver using QIAGEN DNEasy tissue kit. Single nucleotide polymorphism (SNPs) analysis was performed using Sequenom MassARRAY®MALDI-TOF mass spectrometry. All pigs were genotyped for SNPs in innate disease resistance genes including ficolins (*FCN1*, *FCN2*), mannan-binding lectins (MBLs; *MBL1*, *MBL2*), MBL-associated serine protease-2 (*MASP2*), surfactant protein A (*SFTPA*), galectin 4 (*GAL4*), and Toll-like receptors 1, 2, 4 and 5 (*TLR1*, *TLR2*, *TLR4*, *TLR5*). SNPs in some of these genes have been found to be more frequent in pigs with common infectious diseases, including some that are associated with *Salmonella* (Keirstead *et al.*, 2011). Fecal samples were cultured for *Salmonella* at the Animal Health laboratory, University of Guelph (Guelph, ON, Canada). Briefly, fecal samples were placed into tetrathionate (TT) broth and incubated at 41 °C overnight. A loopful of the enrichment media was then streaked onto brilliant green sulfa (BGS) agar plates and incubated overnight at 35 °C. Presumptive *Salmonella* colonies grown on BGS plates were further identified using matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS). The *Salmonella* isolates were serotyped in the *Salmonella* OIE Laboratory for Salmonellosis, Laboratory for Foodborne Zoonoses, Public Health Agency of Canada. A single logistic regression method with farm as random effect was used to analyse the relationship between *Salmonella* shedding and genotype frequency in SNPs in 21 genes.

## Results

*Salmonella* could be recovered from pigs on two farms. On two positive farms, 10 litters tested positive for *Salmonella* with at least one pig per litter shedding *Salmonella*; 4 (57.1 %) and 6 (100 %) litters had at least one pig that tested positive for *Salmonella* shedding. In total, 15 (9.3 %) pigs were identified as shedding *Salmonella*. Serotype analysis determined that the isolates from 6 pigs from 4 different litters on one farm were *Salmonella* Infantis and the isolates from 9 pigs from 6 different litters on another farm were *Salmonella* Worthington. The genotype frequency in 21 genes among pigs with or without *Salmonella* shedding is shown in Table 1. SNP analysis showed an association of *Salmonella* shedding with one SNP in the genes encoding MASP-2 and TLR1 ( $P < 0.05$ ).

## Discussion

It has been shown that the overall immune response varies between different porcine lines (Wilkie and Mallard, 1999). Control of swine infectious diseases (Reiner *et al.*, 2008), in particular susceptibility to *Salmonella* infection in pigs (Galina-Pantoja *et al.*, 2009), by improving genetic resistance has been studied before. Uthe *et al.* (2009) has previously shown that one SNP in a *Salmonella*-infection responsive gene (*CCT7*, chaperonin subunit) associated with circulating neutrophils and monocytes is associated with *Salmonella* shedding in pigs. In our study genotype frequency in a single SNP in both mannan-binding lectins (MBL)-associated serine protease-2 (*MASP-2*) and Toll-like receptor-1 was significantly associated with *Salmonella* shedding. Toll-like receptors (TLRs) play important roles in innate immune responses through inflammatory cytokine production. For example, *TLR1* expression has been shown to increase during *Salmonella* infection in mice so defects in *TLR1*, such as the one in this study associated with *Salmonella* status, could impact the pathogenesis of *Salmonella* infection and/or colonization (Töttemeyer *et al.*, 2005). Further, TLR5 is a pattern recognition receptor that recognizes bacterial flagellin including the flagellin of *Salmonella*, and the presence of one specific SNP in *TLR5* has been shown to be correlated with impaired recognition of *Salmonella* Choleraesuis in pigs (Shinkai *et al.*, 2011). This SNP is specifically distributed in the Landrace and Pietrain breed pigs in Japan and the Czech Republic (Muneta *et al.*, 2012). The Landrace breed is originally from Denmark but is distributed around the world, particularly in Canada, and this specific SNP may be present in Canadian commercial pigs that are progenies of F1 (Yorkshire x Landrace) x Duroc. Further studies are required to determine if SNPs in Toll-like receptors and/or other innate immune genes are involved in resistance against *Salmonella* infection in Landrace and Pietrain breed pigs in Canada as well.

Table 1. Genotype frequency in SNPs among pigs with or without *Salmonella* shedding

SNP <sup>a</sup>	Genotype frequency <sup>b</sup>			SNP	Genotype frequency			
		Sal +	Sal -			Sal +	Sal -	
<b>FCN1_G(1139)A</b>	AA	7.5	0.0	<b>MBL1_C(int)T</b>	CC	78.8	86.7	
	GA	39.7	60.0		CT	21.2	13.3	
	GG	52.7	40.0		<b>MBL2_G(-1081)A</b>	AA	2.1	0.0
<b>FCN2_A(-134)G</b>	AA	98.0	100.0		GA	23.3	13.3	
	GG	2.1	0.0		GG	74.7	86.7	
<b>GAL4_C(96)T</b>	CC	75.9	80.0	<b>MBL2_C(-251)T</b>	CC	18.6	13.3	
	TC	22.1	13.3		CT	55.2	66.7	
	TT	2.1	6.7		TT	26.2	20.0	
<b>GAL4_C(587)T</b>	CC	75.9	73.3	<b>SFTPA_G(439)A</b>	AA	3.5	0.0	
	CT	22.8	26.7		AG	22.8	20.0	
	TT	1.4	0.0		GG	73.8	80.0	
<b>GAL4_C(587)T</b>	AA	28.1	20.0	<b>SFTPA_T(599)A</b>	AA	15.1	13.3	
	AG	46.6	60.0		TA	57.5	40.0	
	GG	25.3	20.0		TT	27.4	46.7	
<b>MBL1_G(271)T</b>	GG	73.8	80.0	<b>TLR1_C(2305)T</b>	CC <sup>c</sup>	74.7	53.3	
	GT	22.8	20.0		TC	24.0	46.7	
	TT	3.5	0.0		TT	1.4	0.0	
<b>MBL1_C(687)T</b>	CC	71.9	86.7	<b>TLR2_C(406)G</b>	CC	95.2	93.3	
	CT	26.7	13.3		GC	4.8	6.7	
	TT	1.4	0.0		<b>TLR4_G(962)A</b>	AA	2.7	6.7
<b>MASP2_C(433)T</b>	CC	23.5	20.0		GA	30.1	33.3	
	CT	64.8	40.0		GG	67.1	60.0	
	TT <sup>c</sup>	11.7	40.0	<b>TLR5_T(834)G</b>	GG	9.0	0.0	
<b>MBL2_T(-2148)C</b>	CC	15.2	13.3			GT	40.0	46.7
	CT	46.9	66.7			TT	51.0	53.3
	TT	37.9	20.0	<b>TLR5_C(1205)T</b>	CC	88.4	93.3	
<b>MBL2_G(-1636)T</b>	GG	26.2	20.0			TC	11.6	6.7
	GT	55.2	66.7		<b>TLR5_C(1919)T</b>	CC	93.8	100.0
	TT	18.6	13.3	CT		6.2	0.0	

<sup>a</sup>SNP nomenclature is described in Keirstead *et al.*, 2011. <sup>b</sup>The homozygous genotypes were compared to the heterozygous genotype for all SNPs except *FCN2\_A(-134)G*, where no heterozygote was identified. For this SNP the homozygous AA was used as the reference. <sup>c</sup>Indicates genotype was significantly different from the reference genotype at  $P < 0.05$  (*MASP2\_C(433)T* TT,  $P = 0.007$ , *TLR1\_C(2305)T* CC,  $P = 0.01$ )

## Conclusion

Increased resistance against *Salmonella* colonization in pigs could reduce food safety concerns by lowering *Salmonella* spread and transmission from farm to fork. These findings indicate that *Salmonella* shedding in pigs is associated with certain genetic variants and suggests that SNPs in innate immune genes could be used to breed for pigs that are more resistant to *Salmonella* colonization and shedding. Further work, including the impact of these variants on production parameters, is required to evaluate the potential to include the identified genetic markers involved in resistance to *Salmonella* infection in swine improvement programs in the swine genetic industry. This study examined *Salmonella* shedding at one point in time, (i.e. at weaning) but further studies are needed to determine SNPs that are associated with carrier stage and intermittent shedding of *Salmonella* in pigs from birth until marketing

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