FOODBORNE PATHOGENS AND DISEASE
Volume 5, Number 2, 2008

DOI: 10.1089/fpd.2007.0071

Seroprevalence of *Trichinella*, *Toxoplasma*, and *Salmonella* in Antimicrobial-Free and Conventional Swine Production Systems

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Abstract

There has been a growing niche-market, outdoor, antimicrobial-free (ABF) swine production system in the last few years prompted by consumers' demand for a more "natural" pork product. The impact of such production systems on reemergence of current and historically significant swine-associated pathogens has not been determined. The objectives of the current study were to determine and compare *Salmonella*, *Toxoplasma*, and *Trichinella* seropositivity in two swine production systems: outdoor ABF and intensive indoor production systems. These three foodborne pathogens represent those with the highest importance for pork consumption. A total of 675 serum samples from three participating states, Wisconsin, North Carolina, and Ohio, were investigated. We found significantly higher seroprevalence of *Salmonella* and *Toxoplasma* from ABF herds (54% and 7%, respectively) than conventional (39% and 1%, respectively) ($p = 0.001$). Two pigs, both from ABF herds, were found to be seropositive for *Trichinella*. The results from this preliminary study suggest that all three pathogens were more commonly present in pigs that were reared in an ABF, outdoor, niche-market type of environment than the conventional, indoor-reared herds though there were some geographical variation in *Salmonella*. This warrants a robust epidemiologic study to determine the role of various risk factors in the two production systems that may lead to persistence of bacterial (*Salmonella*) pathogens and reemergence of parasites (such as *Trichinella*) of historical significance.

Introduction

Swine are implicated as one of the important contributors of foodborne infections in humans. Apart from the common bacterial pathogens, such as *Salmonella*, zoonotic parasitic infestations including *Toxoplasma gondii* and *Trichinella spiralis* are known to have historical significance in swine production. As the pork industry shifted to a more intensive indoor production system complemented with stringent biosecurity measures, the prevalence of these pathogens also declined.

Pigs and undercooked pork are considered an important source of human infection with *Toxoplasma* (Dubey *et al*., 1992; Davies *et al*., 1998; Gamble *et al*., 1999). Prevention and eradication of these diseases in humans is a critical public health goal of the pork industry, veterinarians, and public health authorities. Mead *et al*. (1999) estimated the number of foodborne-related illnesses caused by *Toxoplasma gondii* was more

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than 100,000 cases in the United States alone. In addition, *Toxoplasma* ranks in the top-three leading causes of mortality by foodborne pathogens, predominantly being associated with fetuses and immunocompromised persons.

Toxoplasma gondii is commonly reported in pigs. A report on the national seroprevalence of *T. gondii* in commercial slaughter pigs between 1983 and 1984 with 11,842 serum samples demonstrated about 23% of finishing pigs throughout the United States were serological positive by the agglutination test (Dubey *et al*., 1991). Sows had a twofold higher seroprevalence (46%) as compared to finisher swine (Dubey *et al*., 1991). Based on the seroprevalence survey of pigs including 17 major pig producing states in the United States in 1995 and 2000, the proportion of seropositive breeding females and finisher pigs was dramatically reduced as compared to the previous decade (Patton *et al*., 2002).

During 1997–2001, an outbreak with 33 trichinellosis cases (and no deaths) was reported (Roy *et al*., 2003). While there are many reports in the United States indicated decline in human trichinellosis related of undercooked pork consumptions, occurrence of cases related with wild game meat consumption remains a concern (Gamble *et al*., 1999; Roy *et al*., 2003). *Trichinella* infection from consumption of pork products in the Western World is minimal in this era of modern swine production in intensive production units. On the other hand, in the last few years, the numbers of niche-market, outdoor pig production units are on the rise. Pigs in these facilities are raised with full or partial outdoor access on dirt with open access to soil, vegetation, and wild fauna. The role of these emerging production systems on the potential reemergence of *Trichinella* and other important parasitic diseases has not been investigated.

In contrast to the large survey study of *Toxoplasma gondii* antibody in pigs, the infection rate for *Trichinella* spp. in pigs in the United States is not well determined. Davies and his colleagues reported only 1 out of 2183 (0.057%) pigs reared in indoor production systems in the state of North Carolina was seropositive for *T. spirallis* (Davies *et al*., 1998). The authors concluded that modern swine production systems in concert with good management practices are sufficiently adequate to eradicate *T. spirallis* in swine herds. Of greater concern, there has been a paucity of information on the significance of the rising outdoor niche-market production systems on the rate of *T. gondii* infection in pigs. This study was aimed to investigate and compare the seroprevalence of *Salmonella*, *Toxoplasma gondii*, and *Trichinella spiralis* in pigs reared in two types of production systems; niche-market, outdoor, and antimicrobial-free (ABF) and intensive indoor (conventional) reared herds in three pig-producing states: North Carolina, Ohio, and Wisconsin.

Methods

Sample collection sites

Two types of swine production systems were included in this study: intensive indoor (conventional) and outdoor ABF systems. Under the conventional system of pig production, antimicrobials were added to the feed for growth promotion and were also used for therapeutic purposes. In the extensive ABF pig production system, pigs were reared in open fields in a barricaded area and had free access to the environment including soil and water. In addition, no antimicrobials were used for any purpose other than injections (as needed) for the sows at the farrowing units. Sick pigs that were given antimicrobials for treatment, in the ABF units, were immediately removed from the herd and kept in a different hoop barn or pen enclosure and marketed as conventional and excluded from this study. A total of 675 serum samples from the three participating states—Wisconsin ($n = 351$), North Carolina ($n = 201$), and Ohio ($n = 123$)—were submitted to the Iowa State University Diagnostic Laboratory for analysis. After removing missing and inconsistent (specimens that were not confirmed as from conventional or ABF) data, 616 total data points were further analyzed (Table 1).

Serology

Serological analysis of the parasitic (*Toxoplasma gondii* and *Trichinella spiralis*) and bacterial (*Salmonella*) pathogens using ELISA was conducted at the Iowa State University Diagnostic

Table 1. Number of Samples in the Three Participating States by Production System

State	Production system		Total
	ABF[a]	Conventional	
Wisconsin	245	80	325
North Carolina	74	127	201
Ohio	5	85	90
Total	324	292	616

[a]ABF, antimicrobial-free system.

Center. The standard protocols were followed to detect antibody response against parasitic and *Salmonella* infection. Briefly, diluted test serum samples including negative and positive controls were incubated in the test well to form antigen–antibody complexes. After washing, the anti-swine IgG peroxidase conjugate was then added and incubated to allow the anti-swine IgG peroxidase to bind to the antigen–antibody complex. After a final wash, a chromogen substrate was added to all wells and the plate was read on a plate reader at 450 nm. The cut-off OD used to determine positivity was value of ≥ 0.2 and 0.3 for *Toxoplasma gondii* and *Trichinella spiralis*, respectively, while the positive cut-off of OD% = 25 for detection antibody was evidence of *Salmonella* infection. Seroprevalence of each of the three pathogens was determined and comparison between niche-market ABF and conventionally reared productions were done.

Statistical analysis

SPSS® software package version 15.0 (SPSS Inc., Chicago, IL) was used to carry out by Pearson's chi-square analysis or Fisher's exact test where appropriate. A multivariable logistic regression was also used to determine the potential association of each of the two parasitic pathogens on *Salmonella* seroprevalence (outcome).

Results

Overall the proportion of seropositive animals was 47% (291 of 616), 4.1% (25 of the 616 specimens), and 0.3% (2 of the 616 samples) for *Salmonella, Toxoplasma*, and *Trichinella*, respectively (Table 2). The highest seroprevalence of *Salmonella* was detected from Wisconsin (59% with 95%CI: 54–64) followed by North Carolina (34% with 95%CI: 27–40) and Ohio (34% with 95%CI: 24–44) (Table 2). When comparing the two production systems, we found a significantly higher seroprevalence of *Salmonella* from ABF herds (54%) than conventional (39%) ($\chi^2_{df=1}$, $p = 0.001$) (Table 2).

Seroprevalence of *Toxoplasma* was significantly higher among samples from ABF (6.8%, $n = 22$) than conventional (1.1%, $n = 3$) ($\chi^2_{df=1}$, $p = 0.001$) showing a similar trend as the *Salmonella* seroprevalence (Table 2). Two *Trichinella* seropositive specimens (one each from Wisconsin and North Carolina) were found. Both of these *Trichinella* positive specimens originated from ABF herds. A retesting of both of these positive specimens after 9 months of the initial testing resulted in deterioration of the specimen resulting in lower OD reading (fivefold lower in both cases); one from 1.476 to 0.342 (and 0.374 with third repeat), remaining still within the positive range and the other from 0.387 to 0.074 (and 0.096 with third repeat), which is considered negative.

Table 2. Seroprevalence of *Salmonella*, *Toxoplasma*, and *Trichinella* in Conventional and ABF Swine Production Systems

Pathogen (state)	Total tested (n = 616)		Production system		
	No. positive/no. samples (% prevalence)	95% CI	ABF[a]	Conventional	p value
Salmonella	291/616 (47.2)	43–51	176/324 (54.3%)	115/292 (39.4%)	0.001
Wisconsin	192/325 (59.1)	54–64	150/245 (61.2%)	42/80 (52.5%)	0.1
Ohio	31/90 (34.4)	24–44	5/5 (100%)	26/85 (30.6%)	0.001[b]
North Carolina	68/201 (33.8)	27–40	21/74 (28.4%)	47/127 (37%)	0.2
Toxoplasma	25/616 (4.1)	2–6	22/324 (6.8%)	3/292 (1.1%)	0.001
Trichinella	2/616 (0.3)	0–1	2/324 (0.34%)	0/292 (0%)	0.2[b]

[a]ABF, antimicrobial-free system.
[b]Fisher's exact test.

While at least one sample from North Carolina and Wisconsin was positive for all of the three pathogens, none of the samples from Ohio were positive for *Toxoplasma* and *Trichinella*. We found no significant association between *Salmonella* seropositivity and either of the two parasitic pathogens (*Toxoplasma* and *Trichinella*). There was some evidence of an association between seropositivity for *Toxoplasma* and *Trichinella* (Fisher's exact test, $p = 0.08$). This may be due to the fact that both were more commonly detected from ABF herds but none from conventional ones. Multivariable logistic regression results also supported the univariate findings that there is no association between the occurrence of the two parasitic pathogens and shedding of *Salmonella*.

Discussion

Three globally important zoonotic foodborne pathogens including *Salmonella, Trichinella*, and *Toxoplasma* were studied. Serological analysis of these pathogens was conducted on samples collected from two distinct pig production systems: ABF, outdoor-reared, niche-market type and conventional (indoor-reared). The results from this preliminary study suggest that *Salmonella* and *Toxoplasma* were more commonly present in pigs that were reared in ABF, outdoor, niche-market type of environment than the conventional, indoor-reared herds. This was found to be consistent in all three states—North Carolina, Ohio, and Wisconsin—that were included in the current study.

The high seroprevalence of *Salmonella* in all three states has not been a surprise since the findings indicate prior history of *Salmonella* exposure as compared to current infections when prevalence based on culture and isolation method is used. Consistent with our previous reports, the prevalence of *Salmonella* was higher among ABF herds than conventional ones (Gebreyes *et al*., 2006). A recent report on the *Salmonella* infection demonstrated only 13.2% seroprevalence in 90 Canadian swine farms which was much lower when compared to 41% seoprevalence under conventional setting in our study (Table 2) (Rajic *et al*., 2007).

Another most interesting finding in the current study was the significantly higher seroprevalence of *Toxoplasma* among pigs raised in an ABF environment than those reared in conventional settings. Even though we have not done a detailed risk factor analysis in the current study, the extensive (outdoor) nature of the ABF production system is conducive for exposure of the pigs to various known risk factors for *Toxoplasma* infestations such as cats (the definitive host) and other species that can be harboring cysts in their musculature as compared to the indoor conventional production system. Although the prevalence for *Trichinella* was very low, it was still higher than our expectations (as well as U.S. Department of Agriculture prevalence estimates [Dubey *et al.*, 1992]). The fact that both positive animals were identified among ABF, outdoor-reared swine highlights the potential foodborne risks of these production systems. These pigs are reared in an open-air environment with potential exposure to wild and other domestic fauna, which are the potential sources of this parasite. In addition, the outdoor environment with open access to soil, vegetation, and moisture allows for viable environments for *Toxoplasma* oocysts. The similar trend of higher seroprevalence of *Toxoplasma* and *Trichinella* in outdoor-reared swine was reported in the Netherlands (van der Giessen *et al*., 2007). The frequency distribution of *Toxoplasma* seroprevalence reported in this study ranged from 0.38% in intensive production to 5.62% in outdoor-reared system which was relatively in concordance with our findings (Table 2). According to the National Animal Health Monitoring System's seroprevalence survey reported by Gamble *et al*. (1999), only 0.16% of a total 3048 lactating sows' sera and 0.013% of a total of 7987 gestating/finishing pigs' sera were found to be positive for *Trichinella* spp. antibodies. However, the *Trichinella* spp. seropositive prevalence may vary by the different locations, production types, and management styles; for instance, 15 out of 4078 (0.4%) of serum samples from 156 farms in the northeastern United States were found positive to *Trichinella* spp. antibodies (Gamble and Bush, 1999).

Some evidence of an association between seropositivity for *Toxoplasma* and *Trichinella* ($p = 0.08$) in this study may be due to the fact that both were more commonly detected from ABF herds but none (for *Trichinella*) or rare (for

Toxoplasma) from conventional herds. The finding in this preliminary study warrants the need for a robust epidemiologic study to determine the role of various production-associated risk factors in the two production systems on the safety and wholesomeness of pork products, particularly on the persistence of bacterial (*Salmonella*) and potential reemergence of parasitic (*Trichinella* and *Toxoplasma*) pathogens.

Acknowledgments

This project was funded by a grant from the National Pork Board (NPB-04-108).

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