Similarities in seroprevalence of Toxoplasma gondii, Trichinella spp., Trichuris suis and Ascaris suum in swine in the conventional and antibiotic free swine production systems.

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INTRODUCTION
Helminth parasite infections in swine represent a significant, but understudied health concern for both the swine industry and consumers. While many parasitic infections cause subclinical infections, infected swine pose a public health risk from consumption of contaminated meat products. In swine, infection with parasites has also been shown to negatively impact pig performance and impede weight gain (Kipper et al., 2011; Pittman et al., 2010). Toxoplasma gondii is one of the top pathogens responsible for fatal foodborne infections in the US (Mead et al., 1999). While T. gondii has been recovered from retail pork products, the importance of direct transmission from the consumption of contaminated pork products is still unclear (Dubey et al., 2008). Transmission occurs through ingestion of infected meat containing parasitic, infective cysts (Davies et al, 1998). Trichuris suis and Ascaris suum are both common nematodes identified in swine, with prevalence widely ranging and depending on location and farm practices (Nedjsum et al., 2012). Trichinellosis, while rare in humans, can be associated with consumption of undercooked noncommercial, or antibiotic free (ABF), pork contaminated with Trichinella (Davies et al., 2011). From 2002-2007 there were 54 reported cases of trichinellosis in the US, with 19% of those cases attributed to the consumption of pork products (Kennedy et al., 2009). Nearly half of the worldwide population is infected with one of the above mentioned parasites, with symptoms ranging from diarrhea to malnutrition and occasionally death (Hall et al., 2008). The purpose of this study was to determine the seroprevalence of T. gondii, Trichinella, T. suis, and A. suum in indoor conventionally raised and outdoor Antimicrobial free (ABF) swine.

MATERIALS AND METHODS
Origin of Meat Tissue Fluid Samples
Meat samples were collected as part of a larger longitudinal study on the prevalence of antimicrobial resistant bacteria in conventional and ABF swine production systems in North Carolina (Quintana-Hayashi et al., 2012; Keelara et al., 2013). Briefly, ten cohorts of conventionally-raised pigs and eight cohorts of ABF-raised pigs were sampled five times at farm and at slaughter. One ABF cohort could not be sampled at slaughter and is therefore not represented in the meat tissue samples. Conventionally-raised pigs followed an all-in-all-out production system, where pigs were housed indoors and moved to different farms together as cohorts at different stages of production (farrowing, nursery, and finishing). Conventionally-raised pigs were given antimicrobials for therapeutic and prophylaxis purposes. ABF pigs were raised outdoors on the same farm location for all production stages and were not given antimicrobials for any purpose. At slaughter, all conventionally-raised pigs were sent to a single, large-scale slaughter facility whereas ABF pigs were sent to one of two small-scale slaughter facilities that processed only ABF pigs. Approximately 50 g of meat was taken from each carcass just above the diaphragm after evisceration using a sterile knife, transported on ice, and stored at -20°C until processed. A questionnaire detailing farm management practices was given to producers at each production stage (farrowing, nursery, and finishing). In addition, a similar questionnaire was filled out by lab personnel to document farm conditions and practices while samples were collected.

Serology
Tissue fluids were isolated from 50 g pieces of muscle from individual pigs by freezing at -20°C overnight, thawing at room temperature, and centrifuging at 1g to collect released fluids; tissue fluids were stored at -20°C until use. Tissue fluid samples were tested in duplicate for the presence of antibodies to Trichinella spp. and T. gondii using two 96-well commercial ELISA kits as recommended by the manufacturer (SafePath Laboratories, Carlsbad, CA). The optical density (OD) of each sample was determined at 450 nm using a dual wavelength (450/620 nm) microplate reader (Molecular Devices, Sunnyvale, CA). Specific parasite positive and negative control pig sera supplied by the manufacturer were included on each ELISA plate. ELISA values were reported as the mean optical density (OD) of duplicate wells after subtraction of the OD for the negative control well. Optical densities in both tests which exceeded 0.30 after subtraction of the negative control OD were considered positive.
Tissue fluids were also tested for the presence of specific antibodies to *Trichuris suis* and *Ascaris suum* using 2 in-house ELISAs. *Ascaris* E/S antigens were derived from 72 hour *in vitro* culture of *A. suum* 4th stage larvae (16 days post infection), while *Trichuris* E/S antigens were derived from 72 hour *in vitro* culture of adult worms (42 days post infection) as previously described (Hill et al., 1990). Analysis by spectroscopy to determine the 280:260nm absorbance ratio was used to determine protein concentration. Antigens were passively absorbed onto 96-well microtitre plates, 1μg/well of *T. suis* or *A. suum* excretory/secretory (E/S) antigens, in coating buffer (0.2 M carbonate/bicarbonate buffer, pH 9.6) for 60 minutes at 37°C. Antigen-coated wells were washed three times with wash buffer containing 50 mM Tris, pH 7.4, 150 mM NaCl, 5.0% non-fat milk powder and 0.5% Tween-20 (Tris-NaCl w/T). Tissue fluid was diluted 1:10 in wash buffer without Tween-20 (Tris-NaCl wo/T), and 100μl of diluted tissue fluid was added to each antigen-coated well. Known positive and known negative tissue fluid (diluted 1:10) and serum samples (diluted 1:50) were included on each plate. Plates were incubated for 2 hours at room temperature, then washed 3 times as above with Tris-NaCl w/T. Affinity-purified rabbit anti-swine IgG-peroxidase conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, MD, 1mg/ml), diluted 1:1000 in Tris-NaCl wo/T, was added to each well (100 μl/well) and incubated at room temperature for 2 hours. The plates were washed three times in Tris-NaCl w/T, and rinsed once with distilled water. Chromogen/peroxide substrate was added to each well (100 μl tetramethylbenzidine (TMB), Safepath Laboratories, Carlsbad, CA, USA) and incubated for 10 minutes at room temperature.

The optical density (OD) of each sample was determined at 450nm using a dual wavelength (450/620nm) microplate reader (Molecular Devices, Sunnyvale, CA). ELISA values for each sample were reported as the mean optical density (OD) of duplicate wells after subtraction of the OD for the negative control well. Optical densities in the *Trichuris* ELISA which exceeded 0.30 after subtraction of the negative control OD were considered positive, while an OD which exceeded 0.391 after subtraction of the negative control OD in the *Ascaris* ELISA were considered positive.

**Statistics**

T-tests were used to calculate significant differences in parasite prevalence between farm types (SigmaPlot). Odds ratios were calculated to determine any increased risk associated with various farm management practices, including presence of various animals or pests on farm, anthelminthic administration, herd density, and biosecurity practice. Responses from each stage were combined into an overall farm data set, where a single positive response (i.e. presence of dogs/cats) at any stage marked the overall farm as positive.

**RESULTS**

**Seroprevalence**

A total of 443 meat samples (conventional: 263, ABF: 180) were collected at three slaughter facilities in North Carolina. Meat tissue fluid was tested for serological evidence of *T. gondii*, *T. suis*, *Trichinella spp*, and *A. suum*, Figure 1. Seroprevalence of *T. gondii* and *Trichinella* was low and not significantly different between the conventional (0.7%, 0.3% respectively) and the ABF (2.6%, 2.2% respectively) production systems (P=0.275, P=0.132 respectively). However, swine in the ABF system (77.7%, 132/170) had a significantly higher seroprevalence of *T. suis* than swine in the conventional system (3.0%, 8/263; P<0.001).

In the conventional system, *A. suum* was detected in all cohorts with seroprevalence ranging from 8.7% to 65.5%. Similarly, *A. suum* was detected in all but one ABF cohort, with seroprevalence ranging from 0% to 44%.

**Farm Questionnaire**

No significant associations were detected between presence of parasites and farm questionnaire items, with the exception of *T. suis* and farm type (OR=0, P=0.002) due to 100% of ABF farms being positive for *T. suis*. Risk factors for *A. suum* were difficult to detect due to all but one farm being positive for the parasite. This lack of significant risk factors is likely influenced by the similar answers given on the questionnaires (Table 1). The presence of one parasite was not significantly associated with an increased risk of that farm being positive for a different parasite.
DISCUSSION

While seroprevalence of *T. gondii* and *Trichinella* was higher in ABF compared to conventional swine tissue samples, the difference was not statistically significant. This is likely due to the low number of positive samples. Such low prevalence in the conventional swine system has been reported previously (Davies et al., 1998). Previous studies have detected a significantly higher prevalence of *T. gondii* and *Trichinella* in outdoor, ABF swine compared to conventional swine, some at statistically significant levels (Gebreyes et al., 2008; Giessen et al., 2007). This is likely due to exposure to the outdoor environment in ABF/extensively raised pigs and the presence of biosecurity measures and lack of access to the outdoors in conventional pigs. The seroprevalence of *T. suis* was significantly higher in ABF swine than conventional. This trend has also been seen in previous studies comparing the prevalence of gastrointestinal parasites in different swine management systems (Eijck and Borgsteede, 2005). This may be the result of differences in environmental and farm management factors. Interestingly, we detected a higher, but not statistically significant, seroprevalence of *A. suum* conventional swine compared to ABF. This is in contrast with previous reports that identified a higher prevalence in outdoor and organic swine systems (Eijck and Borgsteede, 2005).

CONCLUSION

These results highlight the similarity of *T. gondii*, *Trichinella*, and *A. suum* seroprevalence rates in conventional and ABF production systems despite significant differences in management practices. While *T. suis* was detected in a significantly higher percentage of ABF swine tissue samples compared to conventional swine, the zoonotic potential of *T. suis* to cause disease in humans is still unclear.

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