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Giardia genotypes in pigs in Western Australia: Prevalence and association with diarrhea.

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Abstract

Little is known of the prevalence of Giardia species and genotypes in pre and postweaned domestic pigs. In the present study, a total of 297 pig fecal samples were screened for the presence of Giardia by PCR and genotyped. An overall prevalence of 31.1% (90/289) (25.8, 36.5 CI) was detected. Giardia was detected in 17% (23/123) (11.8-25.6CI) of pre-weaned piglet faecal samples and 41% (64/156) (33.3-48.7 CI) post-weaned faecal samples analysed. Sequence analysis identified assemblage A and E in pre and post-weaned pigs. Assemblage F was identified in one post-weaned pig. Assemblage E was the most prevalent assemblage detected.

Keywords: Giardia, pigs, pre-weaned, post-weaned, prevalence, assemblage E, diarrhoea, assemblage A, assemblage F.
1. Introduction

*Giardia duodenalis* (syn. *G. lamblia* and *G. intestinalis*) is the most common intestinal parasite of humans and livestock worldwide (Adam, 2001). There are several major assemblages; assemblage AI/AII found in humans, other primates and livestock; assemblage BIII/BIV found in humans and other primates; assemblage C and D, found in dogs; E, found in hoofed livestock; F, found in cats and G, found in rats and mice (Caccio and Ryan 2008).

*Giardia* infections have been reported in pigs in all age groups from nursing piglets to boars and sows from Australia, Asia, Europe and North America with the prevalence ranging between 0.1% and 20%, but infections are not usually associated with clinical illness (Atwill et al., 1997; Guselle et al., 2003; Hamnes et al., 2007; Koudela et al., 1991; Langkjaer et al., 2007; Maddox-Hyttel et al., 2006; Olsen et al., 1997; Sanford, 1987; Xiao et al., 1994).

Little is known of the prevalence of *Giardia* species and genotypes in pigs. The aim of the present study was to determine the prevalence and genotypes of *Giardia* in pre and post-weaned domestic pigs in Western Australia and to determine if there is any association with clinical illness.

2. Material and methods

2.1 Sample Collection

The study was carried out between August and November 2005 and between March and August 2006 in four out of the six large pig producers in Western Australia; 1 indoor (W1) and 3 outdoor
piggeries (G1, WPO, N1) located in the southwest region of Western Australia. A total of 289 faecal samples were collected from pre-weaned piglets (aged 11 days to 3 weeks) (n = 123), post-weaned piglets (aged 4 weeks to 6 months) (n = 156) and sows (n = 10). Samples were collected rectally (from piglets younger than 6 weeks) or within 5-10 minutes of excretion from the ground. Care was taken not to cover the same area more than once and if more than one sample was taken within 1-2m², samples of different consistency or colour were chosen. Samples were placed directly into clean faecal pots at the time of collection and stored in original condition at 4°C as soon as possible after collection (within 24 hours). Faecal consistency was scored at the time of collection from 1 to 4. A faecal score of 1 = diarrhea (soft, unformed stool), 2 = pasty, unformed stool, 3 = soft, formed stool and 4 = hard, dry, formed stools.

2.2 DNA extraction

Genomic DNA was extracted directly from crude faecal samples using a QIAmp DNA stool kit (Qiagen, Hilden, Germany). The DNA was eluted in 50µL of AE buffer to increase DNA concentration and stored at -20°C until required. A negative control (no faecal sample) was used in each extraction group.

2.3 PCR and sequence analysis of the 18S rRNA locus

Amplification of a fragment of the 18S rRNA gene was performed as described by Hopkins et al., (1997) and Read et al., (2002). Giardia duodenalis assemblage B was used as a positive control and a negative control (no DNA) was included for all reactions. The amplified DNA fragments from the secondary PCR product was separated by gel electrophoresis and purified using the
freeze-squeeze method (Ng et al., 2006). A spike analysis (addition of 0.5µl of *G. duodenalis* assemblage B positive control into each sample) was conducted on randomly selected *Giardia* negative samples from each group of DNA extractions (n = 50) to determine if negative results were due to PCR inhibition. Purified PCR products were then sequenced using an ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California) according to the manufacturer’s instructions with the exception that the annealing temperature was raised to 58°C. Nucleotide sequences were analysed using Chromas v2.3 (http://www.technelysium.com.au/chromas.html) edited and compared to existing *Giardia* 18S sequences on GenBank™ using BLAST searches.

2.5 Statistical analysis

The Fisher’s exact test was performed using SPSS 15.0 (Statistical Package for the Social Sciences) for Windows (SPSS inc. Chicago, USA) to determine if there was any association between the prevalence of *Giardia* and *Giardia* genotypes with factors such as faecal score, indoor versus outdoor farms, month of collection and age. Results obtained from samples collected from sows were omitted from the analysis because there were insufficient data for accurate statistical inference. Data for faecal consistency were pooled to create 2 categories comprised of 1 & 2 and 3 & 4. This was done to improve the power of the analysis because there were few data for scores 2 and 3, which may reflect the subjective nature of the scoring system.
3 Results

3.1 Overall prevalence of Giardia in pigs by PCR

Results from testing 289 faecal samples collected from pigs in Western Australia are summarised in Table 1. A total of 31.1% (25.8, 36.5 CI) of faecal samples collected from 289 pigs from Western Australia were shown to be positive for Giardia using PCR. Fifty-seven partial 18S rDNA sequences were obtained from the 90 positives. The results of the sequencing reactions were analysed, edited and compared to existing Giardia 18S rDNA sequences on GenBank™ using BLAST searches. Thirty-five sequences were identified as assemblage E, 17 were assemblage A, two had both A and E and one was assemblage F (Table 1). Spike analysis indicated no inhibition.

3.1 Prevalence in indoor and outdoor herds.

Giardia was found in 30% (77/257) (24.4-35.6 CI) of faecal samples from outdoor herds (G1, WPO and N1) and 40.6% (13/32) (23.6-57.6 CI) of fecal samples from the indoor herd (W1). Giardia positives from the indoor farm (pre and post-weaned) were identified as assemblage E (n=5), assemblage A (n=4) and assemblage F (n=1).

3.3 Prevalence in pre-weaned piglets.

Of the 123 pre-weaned piglet faecal samples analysed, 23 (18.7%) (11.8-25.6CI) were positive for Giardia of which nine (39% of pre-weaned positives) were identified as assemblage
E. Assemblage A was detected in eight samples (34.8% of pre-weaned positives) Two of these samples were mixed A and E infections. The PCR products of the remaining six *Giardia* positive samples had insufficient DNA for sequencing.

### 3.4 Prevalence in post-weaned piglets

Of the 156 post weaned faecal samples analysed, 64 (41%) (33.3-48.7 CI) were positive for *Giardia* (Table 1). Of these, 27 (42% of positive post-weaned pig samples) were identified as assemblage E and 11 (17% of positive post-weaned pig samples) were identified as Assemblage A. One isolate was assemblage F and the remaining 25 *Giardia* positive samples isolated from post-weaned pigs were unable to be identified due to insufficient DNA. *Giardia* was detected in three out of ten faecal samples collected from sows.

### 3.5 Statistical analysis.

There was no statistical difference in the proportion of samples positive for *Giardia* from different farms and from different farming systems between different months of collection (p<0.05). Assemblage E was the most prevalent genotype of *Giardia* observed (p<0.05). The higher prevalence of *Giardia* in post-weaned pigs was statistically significant (p < 0.05). There was also a significant association between the genotype of *Giardia* detected and faecal consistency. A higher proportion (91.7%) of faeces from post-weaned pigs containing Assemblage E were categorised as score 1&2 (soft to diarrhoeic stool) compared to score 3&4 (soft-hard, formed stool) (p<0.05). A higher proportion (90%) of faeces from post-weaned pigs containing Assemblage A were categorised as score 3&4 compared to score 1&2 (p<0.05).
Discussion

*Giardia* infections appear to be quite common in pigs, and have been found in all age groups from nursing piglets to boars and sows (Guselle and Olson, 1999 and Maddox-Hytte et al., 2006). In the present study, *Giardia* was detected in 31.1% (90/289) of domestic pigs from Western Australia. This is much higher than a previous study, which reported a prevalence of 0.8% in domestic pigs in Western Australia (Ryan et al., 2003). The higher positive rate in the present study is likely due to the fact the all samples were screened by PCR whereas the previous study relied on microscopy for initial identification of *Giardia* and studies have shown that PCR detects 5 times more *Giardia* positives than microscopy (Ryan et al., 2005). As there are only six large pig producers in Western Australia and four were sampled, the results of the present study are representative of the pig industry in Western Australia.

Few studies have examined the prevalence of *Giardia* in pre-weaned versus postweaned piglets. In the US, *Giardia* was detected in 1.5% (3/24) of sows, 8.3% (8/96) of preweaned piglets and 2.6% (8/32) of postweaned pigs (Xiao et al., 1994). In Denmark, *Giardia* was detected in 4% (10/245) of sows, 3% (15/488) of preweaned piglets and 38% (190/504) of postweaned piglets (Maddox-Hytte et al., 2006). In the present study, *Giardia* was detected in 17% of preweaned animals, 32.2% of postweaned animals and 20% of sows. Assemblage E was the most common *Giardia* genotype and was detected in 64% of genotyped pre-weaned isolates and 67% of genotyped post-weaned isolates. Assemblage A was detected in 36% and 33% of pre and post-weaned isolates respectively. In Denmark, assemblage E was also the most common genotype and was identified in 62% of genotyped postweaned pigs and assemblage A was
detected in 12% of post-weaned pigs. Only one isolate from pre-weaned pigs was genotyped and this was Assemblage A. In that study, assemblage D was identified in one post-weaned pig and a novel genotype, K2521 (DQ182604) was identified in a post-weaned pig, which grouped with assemblage E at the conserved 18S locus but was genetically distinct at the more variable gdh locus (Langkjaer et al., 2007). In the present study, assemblage F was identified in one isolate from a post-weaned pig from the indoor farm (W1). Assemblage F is thought to be feline specific but has been reported in human isolates from Ethiopia (Gelanew et al., 2007). We were unable to sequence the isolate at another locus to confirm the identification of assemblage F. It is also possible that the assemblage F cysts were passively passed to the pig by an infected cat.

In the present study, the presence of assemblage E in pigs was significantly associated with a fecal score of 1&2 (soft to diarrhoeic stool) (p<0.05), whereas assemblage A was not. This is in contrast to previous studies which have reported no association between *Giardia* infections in pigs and diarrhoea (Xiao et al., 1994 and Koudela et al., 1991; Maddox-Hyttel et al., 2006; Hamnes et al., 2007; Langkjaer et al., 2007). In the present study, viral and bacterial infections were not screened for therefore it is possible that *Giardia* was not the cause of diarrhoea. Larger numbers of samples over a longer time-period need to be examined for a wide range of pathogens to determine if *Giardia* is a cause of diarrhoeal illness in pigs. However the results of the present study suggest that *Giardia* is a common pathogen in pigs and may be an important cause of diarrhoeal illness.
References


Ryan, U.M., Bath, C., Robertson, I., Read, C., Elliot, A., McInnes, L., Traub, R., Besier, B. 2005. Sheep may not be an important zoonotic reservoir for *Cryptosporidium* and *Giardia* parasites. Applied and Environmental Microbiology 71, 4992-7.

Table 1. Prevalence of *Giardia* sp. in pigs of different age-groups in Western Australia (95% confidence intervals in parantheses). ND = Not done. NA = not applicable.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Type</th>
<th>Pig class</th>
<th>Not tested</th>
<th>No. positive</th>
<th><em>Giardia</em> Prevalence %</th>
<th>A</th>
<th>E</th>
<th>F</th>
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<tr>
<td>WPO</td>
<td>Outdoor</td>
<td>Preweaner</td>
<td>41</td>
<td>3</td>
<td>7.3 (0, 15.3)</td>
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<td></td>
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<td>Postweaner</td>
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<td>19</td>
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<td>3</td>
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<td></td>
<td></td>
<td>Sow</td>
<td>9</td>
<td>2</td>
<td>22.2 (9, 49.4)</td>
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<td>ND</td>
<td>ND</td>
</tr>
<tr>
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<td>Preweaner</td>
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<td>ND</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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<td></td>
<td></td>
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<td></td>
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<td>ND</td>
<td>-</td>
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<tr>
<td>WI</td>
<td>Indoor</td>
<td>Preweaner</td>
<td>14</td>
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<td>1</td>
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<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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<td></td>
<td>289</td>
<td>90</td>
<td>31.1 (25.8, 36.5)</td>
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