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Prevalence and Identification of *Cryptosporidium* spp. from Swine Slurry

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ABSTRACT

Cryptosporidium spp. were detected in 17 of 135 swine lagoon samples from five farms by 18S ribosomal DNA locus and PCR. Seventeen positive samples identified were included two distinctive genotypes *C. suis* and *Cryptosporidium* sp. based on a small-subunit rRNA gene-based PCR-restriction fragment length polymorphism analysis. *Cryptosporidium* spp. were detected out of farrowing, farrowing and nursery (mix), and finishing. Prevalence rate was 12.6% with infection rates between 3.7 and 18.5%. We concluded that *Cryptosporidium* oocysts can persist in treated lagoon and potentially contaminate surface water through improper discharge. This study was undertaken for the evaluation of the infection status of the genotypes of *Cryptosporidium* spp. in swine lagoon.

Keywords: *Cryptosporidium*, prevalence rate, *C. suis*, *Cryptosporidium* sp., swine slurry

I. Introduction

Cryptosporidium were first recognized by Tyzzer (1907) in the gastric glands in mice.[1] *Cryptosporidium* is an obligate intestinal protozoa belonging to the phylum Apicomplexa. *Cryptosporidium* spp. are enteric parasites that occur in many animal species including pigs. *Cryptosporidium* spp. is the most important cause of infection in mammals including humans and associated with the human immunodeficiency syndrome.[2] *Cryptosporidium* infection has been investigated in many countries throughout the world since first described by Kennedy *et al.*(1977).[3-6] *Cryptosporidium* sp. oocysts can sustain for long periods outside host in moist environments such as swine lagoon and widely distribute in the environment. Swine lagoon is products of wastewater treatment containing organic and inorganic compounds. Cryptosporidiosis may be of economic concern and cause disorders of the gastrointestinal systems in piggeries.[6,7]

Molecular methods for the identification of *Cryptosporidium* have been developed and applied in studies for the occurrence of the organisms. Thirteen *Cryptosporidium* spp. have been recognized by this time : *C. parvum, C. hominis, C. muris, C. andersoni, C. felis, C. wrairi, C. baileyi, C. meleagridis, C. canis, C. suis, C. molnari, C. saurophilum,* and *C. serpentis.* Four pig genotypes, *Cryptosporidium* sp., *C. suis, C. parvum* including pig genotype II, have been genetically described until now, but *C. suis* and *Cryptosporidium* sp. are detected most commonly in pigs.[8,9]

This study was undertaken for the evaluation of the infection status of the genotypes of *Cryptosporidium* spp. found in swine slurry in Atlanta.

II. Materials and Methods

All the lagoon samples of pigs were obtained from farrowing, farrowing & nursery, and finishing units on five pig farms in Atlanta during Spring-Summer, 2008. Each untreated samples of 500 m*l* were collected in the plastic container from piggeries, and transported at the cool temperature to the microbiology and immunology laboratory for analysis. The samples were kept in the cool room between

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sampling and processing at the laboratory. A total of 135 samples from swine lagoon were collected and analyzed for the present of *Cryptosporidium* spp.

Swine lagoon samples were carried out sieving, centrifugation and sugar gradients.[10] DNA from *Cryptosporodium* oocysts was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany) and analysed by polymerase chain reaction (PCR) of the SSU ribosomal RNA gene.[11] DNA was stored frozen at −20°C before PCR. DNA extraction and PCR amplication of the diagnostic region of the small subunit ribosomal RNA gene (SSUrRNA) using primers.[11,12]

PCR reaction was carried out in 50 μl containing 5 μl of buffer, 200 μl of each DNA, 10 pmoles of each primer, 20 μg nonacetylated BSA and 1.25 U of tag polymerase. In the primary PCR, each PCR mixture, total volume of 100 μl was contained 10 μl 10PCR buffer, 6 mM $MgCl_2$, 200 uM each dNTP, forward and reverse primers at a concentration of 200 nM each, 400 ng/μl of non-acetylated BSA, 2.5 U tag polymerase, and 0.5-3.0 μl DNA template. A total of 35 cycles consisting of : 94°C for 45 sec, 55°C for 45 sec, and 72°C for 60 sec make up the PCR program. An initial hot start was carried out at 94°C for 3 minutes and final extension at 72°C for 7 minutes. For the secondary PCR, the following primers were used as forward and reverse primers, respectively : 5'-GGAAGGGTTGTATTTATTAGA-TAAAG-3', and 5'-CTCATAAGGTGCTGAAGGAGTA-3'.

III. Results and Discussion

PCR products (SSR rRNA) that were of the expected size were obtained from 17 of the 135 (12.6%) lagoon samples collected from five farms in Atlanta (Table 1). In a detailed examination of the localization of *Cryptosporidium* oocysts in five farms, they revealed that four Boiling, three Wilson, four Yeargin, five Bridges and one out of Kakega farms respectively, were positive for *Cryptosporidium* spp. One hundred thirty five PCR products from each farm were sequenced. The results of DNA sequencing confirmed that PCR products were from two *Cryptosporidium* species (Table 2).

Fifteen PCR products from four farms were yielded sequences identical to each other. The sequence found in the GenBank in this study was deposited under accession number AF115377 of *C. suis* and accession no. EF012375 of *Cryptosporidium* sp. (Table 3). The PCR products from Boling and Kakega farms were generated *Cryptosporidium* sp.. *Cryptosporidium* sp. was detected by PCR, observing the presence in the lagoons of farrowing and finishing due to their opportunism nature of this protozoa. Molecular characterization, sequence analysis of regions of the 18s rRNA were demonstrated distinct genotypes for samples obtained from swine lagoon. They have helped to clarify *Cryptosporidium* taxonomy and validate the existence of species, compared to the traditional methods such as host range, oocyst morphology and site of

Table 1. Prevalence of *Cryptosporidium* infections on five pig farms

Farm	Group	No. sampled	No. positive	Prevalence (%)
Boiling	Finishing	27	4	14.8
Wilson	Finishing	27	3	11.1
Yearing	Finishing	27	4	14.8
Bridges	Farrowing & nursery	27	5	18.5
Kakega	Farrowing	27	1	3.7
Total		135	17	12.6

Table 2. Prevalence of *C. suis* and *Cryptosporidium* sp. according to species

	Boiling	Wilson	Yeargin	Bridges	Kakega	Total
C. suis	3	3	4	5	0	15
Cryptosporidium sp.	1	0	0	0	1	2
Total	4	3	4	5	1	17

Table 3. *Cryptosporidium* species isolated from swine lagoon of five farms

Sample (Farms)	Genotyping SSU-rRNA gene	Accession number
Boling 1	*C. suis*	AF115377
Boling 2	*C. suis*	AF115377
Boling 3	*C. suis*	AF115377
Boling 4	*Cryptosporidium* sp.	EF012375
Wilson 1	*C. suis*	AF115377
Wilson 2	*C. suis*	AF115377
Wilson 3	*C. suis*	AF115377
Yeargin 1	*C. suis*	AF115377
Yeargin 2	*C. suis*	AF115377
Yeargin 3	*C. suis*	AF115377
Yeargin 4	*C. suis*	AF115377
Bridges 1	*C. suis*	AF115377
Bridges 2	*C. suis*	AF115377
Bridges 3	*C. suis*	AF115377
Bridges 4	*C. suis*	AF115377
Bridges 5	*C. suis*	AF115377
Kakega 1	*Cryptosporidium* sp.	EF012375

infection.[2,13] A diagnosis of *C. suis* was confirmed by sequencing strain p1 18S ribosomal RNA gene and finding *Cryptosporidium* sp. with the BG5 small subunit ribosomal RNA gene. According to sequence analysis, 11 finishing, 5 farrowing & nursery (mixing), and 1 farrowing carried *Cryptosporidium* oocysts. *C. suis* infections occurred in finishing and farrowing & nursery, while *Cryptosporidium* sp. infections occurred in farrowing and finishing. Results in this study revealed that pigs are susceptible to *C. suis* infection. By this time, four *Cryptosporidium* sp. have been isolated from pigs ; *C. suis, Cryptosporidium* genotype ll, *C. muris* and *C. parvum*.[6,14] Finishing of Boling farm carried mixed infections of *Cryptosporidium* sp. and *C. suis*.

The traditional methods such as host range, oocyst morphology and site of infection do not provide a reliable method for the *Cryptosporidium* species identification. The method for oocyst detection or variation in the groups surveyed are likely to influence in the prevalence of *Cryptosporidium* species when compared with other studies.[15] Molecular methods using PCR-RFLP analysis have been used to screen clones to select products for sequencing.[13] According to morphology by Upton and Current, 1985, oocysts were classified as belonging to *C. suis* and *Cryptosporidium* sp. The PCR-RFLP which uses for heterologous regions in the SSU gene fragement, is a useful tool for differentiating *C. suis* and *Cryptosporidium* spp.[16]

The prevalence ranged between 3.7 and 18.5% on a swine lagoon in the five piggeries, and the results of this study suggest that there is an association between diarrhoea and *Cryptosporidium* in piggeries. Diarrhoea caused by *Cryptosporidium* spp. has been a major problems in pigs worldwide.[9] Morgan *et al.*[17] reported that *C. suis* and *Cryptosporidium* sp. are accompanied with acute diarrhoea in natural outbreaks of *Cryptosporidium* in pigs and contributed to diarrhoea in suckling pigs. This finding suggests that *Cryptosporidium* may contribute to diarrhoea in farrowing and finishing pigs.

The prevalence of *Cryptosporidium* in this study was 12.6%, which was higher than 9.1% positive litters found by Xiao *et al.*,[18] 5.3% in Ontario,[19] but lower than 22.0% found by Maddox-Hyttel *et al.*[20] This result was similar to that of 11% in Canadian study by Olsen *et al.*.[21] Prevalence rates were higher in finishing and farrowing & nursery than in farrowing farm. *Cryptosporidium* infections are probably because of physical features of facilities on the farms that oocysts can remain viable and infectious such as swine lagoon. The oocysts may sustain for extended periods due to the humid environment.[22] *Cryptosporidium* species oocysts from the waste materials in an underfloor pit, pumped into a lagoon for anaerobic digestion using effluent. The prevalence of *Cryptosporidium* species oocysts infection is probably due to the presence of carriers, as well as to the features of swine slurry where oocysts could remain viable and prepatent infections. The well-managed and better hygienic measures may reduce the prevalence of *Cryptosporidium* infection in piggeries.

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