

Age-related Infection with *Cryptosporidium* Species and Genotype in Pigs in China*

YIN Jian Hai^{1,△}, YUAN Zhong Ying^{1,△}, CAI Hui Xia², SHEN Yu Juan^{1,#}, JIANG Yan Yan¹,
ZHANG Jing¹, WANG Yan Juan¹, and CAO Jian Ping^{1,#}

1. National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention; Key Laboratory of Parasite and Vector Biology, MOH, China; WHO Collaborating Center for Malaria, Schistosomiasis and Filariasis, Shanghai 200025, China; 2. Qinghai Institute for Endemic Disease Prevention and Control, Zong Zhai, Xining 811602, Qinghai, China

Abstract

Objective Pigs, as hosts of zoonotic *Cryptosporidium* species/genotypes, are domestic animals with public health significance. The present study was to characterize the infection rate and species/genotype of *Cryptosporidium* in pre-weaned and post-weaned pigs from Shanghai and Shaoxing, China.

Methods A total of 208 fecal samples (42 from pre-weaned piglets, and 166 from post-weaned pigs) were examined by nested PCR of the 18S rRNA gene and analyzed by phylogenetic DNA fragment sequencing of secondary PCR products.

Results Infection was detected in 79 samples (19/42 pre-weaned piglets, and 60/166 post-weaned pigs). *C. suis* (14/79) and *Cryptosporidium* pig genotype II (65/79) were identified; piglets were more susceptible to the former (13/14) and post-weaned pigs to the latter (59/65).

Conclusion Infection of *Cryptosporidium* spp. in pigs was age-specific; piglets were more susceptible to *C. suis* while pigs were more susceptible to *Cryptosporidium* pig genotype II. These findings combined with the isolation of the two *Cryptosporidium* from water suggest that pigs may be a source of zoonotic *Cryptosporidium* water pollution. Improvements in pig feeding practices, sewage discharge, feces disposal and field worker protection are therefore important to prevent potential public health problems.

Key words: *Cryptosporidium suis*; *Cryptosporidium* pig genotype II; Age-specificity; Pigs; China

Biomed Environ Sci, 2013; 26(6):492-495

doi: 10.3967/0895-3988.2013.06.010

ISSN:0895-3988

www.besjournal.com(full text)

CN: 11-2816/Q

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INTRODUCTION

Cryptosporidium infection is a major cause of diarrhea worldwide, severely affecting both humans and animals. Natural

infection in pigs is predominantly with *C. suis* or *Cryptosporidium* pig genotype II^[1-4], though other species such as *C. parvum*, *C. muris* and *C. andersoni* have been reported^[5-7]. All of these species and genotypes are zoonotic^[8-9] and represent potential

*This work was supported by grants from the Chinese Special Program for Scientific Research of Public Health (No. 200802012) and the National S&T Major Program of China (No. 2009ZX10004-201 and No. 2012ZX10004-201).

△These two authors contributed equally to this work.

#Correspondence should be addressed to SHEN Yu Juan and CAO Jian Ping. Tel: 86-21-64735258, 86-21-64377008-2407. Fax: 86-21-64332670. E-mail: caojp@yahoo.com, amyshyj12@163.com

Biographical note of the first authors: YIN Jian Hai, male, born in 1984, majoring in molecular epidemiological study of emerging parasitic diseases, E-mail: chart2543@163.com; YUAN Zhong Ying, female, born in 1971, associate professor, majoring in detection and diagnostics of parasitic diseases, E-mail: yuanzhy0606@yahoo.cn

Received: June 12, 2012;

Accepted: October 23, 2012

threats to public health.

China is the world's largest producer and consumer of pork. The Yangtze River region and northern China are the key pork producing and internal distribution areas. The cities of Shanghai and Shaoxing in Zhejiang province are located in the Yangtze River Delta, which is the most developed part of the Chinese mainland, and one of the most densely populated areas. The high population may increase the risk of spread of infection with micro-organisms that are carried in the feces of domestic animals, including *Cryptosporidium*, and can be passed to humans. *Cryptosporidium* infection in pigs in this area has been reported in several publications^[10-12], but age-related rates of infection have not been determined. We used molecular methods to identify *Cryptosporidium* species and genotype infection in two age groups of pigs in Shanghai and Shaoxing.

MATERIALS AND METHODS

Samples

Two hundred and eight fecal samples were collected randomly from pigs on six separate pig farms in Shanghai and one in Shaoxing between April 2009 and July 2011. And all of the samples were from different individuals confirmed by the information of pigs. The samples were collected immediately after defecation and placed in individually labeled plastic bags. In a previous publication, 94 samples from post-weaned pigs had been analyzed^[12]. In the present study, we extended the pigs' age range to include pre-weaned piglets. The weight of the samples were approximately 20 g for the pre-weaned piglets and 50 g for the post-weaned pigs.

DNA Extraction, Amplification, and Sequencing

Approximately 20 g of each sample was concentrated by filtration and centrifugation, and the sediments were stored at -70 °C until use^[12]. Genomic DNA was extracted using a QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions, with one minor adjustment, in which the fecal suspension was heated for 10 min at 95 °C, and then the supernatants containing DNA were stored at -20 °C until amplification.

Nested polymerase chain reaction (PCR) amplification of the *Cryptosporidium* 18S rRNA gene

fragment was performed as described previously^[12-13]. Secondary PCR products were analyzed by 2% agarose gel electrophoresis and ethidium bromide staining. Positive secondary PCR products were subjected to two-directional sequencing with secondary primers by Shanghai Biotechnology Co. Ltd. (Shanghai, China). Phylogenetic analysis of the relationship between the sequences obtained in the present study and sequences acquired from GenBank was performed using MEGA 4.1 software^[14].

Data Analysis

Data were calculated and analyzed using Microsoft Excel 2010 software. Differences in distribution were evaluated using the chi-square (χ^2) test. $P < 0.05$ was considered statistically significant.

RESULTS

One-hundred and thirty-four samples (22 from pre-weaned piglets and 112 from post-weaned pigs) were collected from Shanghai and 74 (20 pre-weaned piglets and 54 post-weaned pigs) from Shaoxing (Table 1). On nested PCR analysis with positive and negative controls, *Cryptosporidium* infection was found in four pre-weaned and 35 post-weaned samples from Shanghai and in 15 pre-weaned and 25 post-weaned samples from Shaoxing (Table 1). The difference in infection rate between the two age groups was not statistically significant ($\chi^2 = 1.78$, $0.25 < P < 0.5$); thus, the risk to *Cryptosporidium* infection was similar in piglets and pigs (Table 2).

A total of 79 samples were found to be positive, but only *C. suis* and *Cryptosporidium* pig genotype II were isolated. No mixed infections with the two were found. Thirteen pre-weaned samples and one post-weaned sample contained *C. suis*, and six pre-weaned samples and 59 post-weaned samples contained *Cryptosporidium* pig genotype II (Table 3).

Table 1. The Source and Age-related Distribution of Pig Stool Samples

Age Group	Shanghai		Shaoxing	
	Total	Positive	Total	Positive
Pre-weaned	22	4	20	15
Post-weaned	112	35	54	25
Total	134	39	74	40

Table 2. Age-related Infection with *Cryptosporidium* in Pigs

Age Group	Positive	Negative	Total
Pre-weaned	19	23	42
Post-weaned	60	106	166
Total	79	129	208

Note. $\chi^2=1.78$, $0.25 < P < 0.5$, $\alpha=0.05$.

Table 3. Age-related Distribution of *Cryptosporidium* Species and Genotype Infection

Age Group	<i>C. suis</i>	<i>Cryptosporidium</i> Pig Genotype II	Total
Pre-weaned	13	6	19
Post-weaned	1	59	60
Total	14	65	79

Note. $\chi^2=44.10$, $P < 0.005$, $\alpha=0.05$.

These differences in species/genotype infection between the two age groups were significant ($\chi^2=44.10$, $P < 0.005$).

The nucleotide sequences in the present study were deposited in GenBank under accession numbers JQ936482-JQ936508.

DISCUSSION

Cryptosporidium infection has been identified in more than 240 animal species as well as in humans, in whom it is an important cause of diarrhea. There are at least eleven zoonotic *Cryptosporidium* species^[15-18] and several zoonotic genotypes^[8,19]. *C. suis* and *Cryptosporidium* pig genotype II are the most common infection in pigs.

In 2010, almost 50% of the world's pork production came from China^[20]. Shanghai and Shaoxing in the Yangtze River Delta are key pork production regions, where zoonotic *Cryptosporidium* has been isolated in pigs^[10-12]. Moreover, *C. suis* has been found in waste, source and tap water in Shanghai^[21-22], and we have identified *Cryptosporidium* pig genotype II in water from Shanghai and Shaoxing (data unpublished). Cryptosporidiosis is a common waterborne disease in humans, and the proximity to water of people living in the delta, together with frequent population movements, increase the risk of exposure, leading to a potential public health problem. Understanding the *Cryptosporidium* infection status of pigs and its

age and species/genotype specificity is therefore essential.

In the present study, only *C. suis* and *Cryptosporidium* pig genotype II were identified in pre- and post-weaned pigs from Shanghai and Shaoxing. This is similar to the findings of Henan^[4], but the infection rate in our study is higher ($\chi^2=149.06$, $P < 0.005$). Our infection rate was also higher than that observed in Denmark ($\chi^2=18.24$, $P < 0.005$)^[23], but was not statistically different from that in Australia ($\chi^2=2.41$, $0 < P < 0.25$)^[3]. The present study suggests that both *C. suis* and *Cryptosporidium* pig genotype II are age-specific, which differs from the reports of Jeníková^[24] and Suárez-Luengas^[25]. The mechanisms of resistance of piglets to *Cryptosporidium* pig genotype II and of post-weaned pigs to *C. suis* remain unclear.

In conclusion, 19 of 42 piglets and 60 of 166 pigs were found to be infected with *Cryptosporidium*. The infection was age-specific; piglets were more susceptible to *C. suis* and post-weaned pigs were more susceptible to *Cryptosporidium* pig genotype II. Combined with the isolation of this species/genotype from water in Shanghai and Shaoxing, these findings suggest that pigs may be a source of zoonotic *Cryptosporidium* water pollution. Improvements in pig feeding practices, sewage discharge, feces disposal and field worker protection are therefore important to avoid public health problems in the future.

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