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Short communication

Prevalence and age-related infection of *Cryptosporidium suis*, *C. muris* and *Cryptosporidium* pig genotype II in pigs on a farm complex in the Czech Republic

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ABSTRACT

A total of 413 pig faecal samples were collected from pre-weaners (119), starters (131), pre-growers (123) and sows (40) from a farm with a closed breeding system segmented into two breeding complexes and a growing complex in the region of Vysočina, Czech Republic and screened for the presence of *Cryptosporidium* using staining methods and genotyping (SSU rRNA). *Cryptosporidium* oocysts were detected by microscopy in the faeces of 21.1% of the samples (87/413). Sequence analyses and RFLP identified *C. suis* in 44, *Cryptosporidium* pig genotype II in 23 and *C. muris* in 2 samples. No mixed infections were found.

Pigs under 7 weeks of age were infected with *C. suis* only. *Cryptosporidium* pig genotype II was found in animals from 7 weeks of age. No relationship was found between diarrhoea and any *Cryptosporidium* infection in any of the different age groups ($P < 0.05$). The pre-weaned pigs shed significantly more *Cryptosporidium* oocysts than older pigs and it was associated with *C. suis* infection.

1. Introduction

Cryptosporidiosis in pigs has been reported worldwide (see Johnson et al., 2008). Infection with *C. suis* and the *Cryptosporidium* pig genotype II are reported most frequently, nevertheless pigs can be naturally infected by another four *Cryptosporidium* species/genotypes, namely *C. parvum*, *C. muris*, *Cryptosporidium* mouse genotype I and the *Cryptosporidium* sp. Eire w65.5 isolate (Morgan et al., 1999; Ryan et al., 2003, 2004; Xiao et al., 2006; Chen and Huang, 2007; Zintl et al., 2007). Recently, some studies in pigs have indicated age specificity of various *Cryptosporidium* species/genotypes, as it is known in cattle (Langkjaer et al., 2007; Johnson et al., 2008).

The aim of the present study was to document the prevalence of *Cryptosporidium* infection in pigs on farms with a closed breeding system in the Czech Republic and to determine the age specificity of various *Cryptosporidium* infections.

2. Materials and methods

We conducted a coprological survey of *Cryptosporidium* on a randomly selected farm in the region of Vysočina, Czech Republic during the year 2007. The selected farm had three units with a closed breeding system: two breeding complexes and a growing complex.

Each breeding complex was divided into two sections: one with individual pens for farrowing sows and their litters where piglets (pre-weaners) stay until weaned at 4 weeks of age. The second section was adjacent and composed of large communal pens for weaned piglets (starters), where they were kept until reaching 8 weeks of

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age. Thereafter, piglets were transferred to the growing complex (pre-growers), where they were kept until about 12 weeks of age. Faeces (413) were collected from animals of different age categories: pre-weaners ($n = 119$), starters ($n = 131$), pre-growers ($n = 123$) and sows ($n = 40$). The samples were collected from the floor immediately after defecation into individually labelled sterile tubes and stored at 4 °C until processed in the laboratory (within 24 h) using the aniline-carbol-methyl violet staining method (Miláček and Vítovec, 1985). Faecal consistency was noted at the time of sampling. Infection intensity was determined as a number of oocysts per gram (OPG) according to Kváč et al. (2007).

Genomic DNA was isolated from all *Cryptosporidium* positive samples as described previously (Sak et al., 2008). An approximately 830 bp long fragment of the SSU rRNA gene of *Cryptosporidium* isolates was amplified by nested PCR protocol according to Jiang et al. (2005). The secondary PCR products were sequenced using ABI BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and ABI3130 Genetic Analyzer (Applied Biosystems). The nucleotide sequences were analyzed using Chromas Pro v1.32 (www.technilysium.com.au/chromas.html) and aligned with reference sequences using ClustalX (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/). In addition, *Cryptosporidium* species and genotypes were determined by nested PCR of a SSU rRNA gene fragment and RFLP analysis with the endonucleases *Ssp*I and *Vsp*I (Fermentas) as described previously (Xiao et al., 2001).

The Chi-square test and Student's *t*-test were used to evaluate the differences in prevalence and infection intensity, respectively. Statistical tests were calculated using Statistica®, Release 5.1 Software (Statsoft, Tulsa, OK, USA, 1997).

3. Results

Cryptosporidium infection was found in all examined age categories. A total of 21.1% of the faecal samples were microscopically positive for *Cryptosporidium* oocysts. From the 87 positive samples 69 partial SSU rRNA sequences were obtained, the other 18 *Cryptosporidium* positive samples had insufficient DNA for PCR and sequencing (Table 1). Forty five sequences were identified as *C. suis* (GenBank Accession No. AF115377), 22 were identified as *Cryptosporidium* pig genotype II (GenBank Accession No. DQ182600), 2 as *C. muris* (GenBank Accession No. EU245044), and no *Cryptosporidium* mixed infections were recorded. At the time of collection, most animals appeared to be in good health condition, and in 26 cases diarrhoea was inferred from the liquid consistency of the faeces [pre-weaners ($n = 9$), starters ($n = 9$), pre-growers ($n = 8$)]. From these animals, only one starter had *Cryptosporidium* pig genotype II. Results of statistic analysis did not reveal association between the occurrence of diarrhoea and *Cryptosporidium* species/genotype infections in pigs ($P > 0.05$). Tests were not conducted to detect enteric pathogens other than *Cryptosporidium*.

Infection intensity varied from 2×10^2 to 1×10^6 oocysts per gram of faeces (OPG). *Cryptosporidium* infection intensity was less in older pigs. The mean infection intensity and the maximum OPG were 215,159 and 1,170,000 in pre-weaners, 25,620 and 373,000 in starters, and 693 and 1700 in pre-growers ($P < 0.01$), respectively. Pigs infected with *C. suis* shed a statistically higher number of oocysts (mean 72,729 OPG) compared with *Cryptosporidium* pig genotype II (mean 552 OPG) ($P < 0.01$).

Of the 119 pre-weaned pig faecal samples 26 were positive for *Cryptosporidium*, 18 (69%) of them were identified as *C. suis* and no other *Cryptosporidium* was identified in the samples. Of the 38 positive samples in starters 26 (68.4%) were identified as *C. suis*, 5 (13.2%) as *Cryptosporidium* pig genotype II, and 1 (2.6%) as *C. muris*. Of the total 123 faecal samples from pre-growers 21 (13.9%) were *Cryptosporidium* positive. Of these, 17 (81%) were identified as *Cryptosporidium* pig genotype II, one (4.8%) as *C. suis* and one (4.8%) *C. muris* (Table 1).

While *C. suis* infection was recorded only during the first 7 weeks of age, the *Cryptosporidium* pig genotype II was found from 7 weeks of age, except for sporadic findings of *C. muris* at 6 and 10 and *C. suis* at 10 weeks of age, respectively (Fig. 1).

4. Discussion

Pigs of a wide age range are susceptible to *Cryptosporidium* infections and the overall prevalence recorded from many countries ranged from 6 to 60% (Quílez et al., 1996; Wieler et al., 2001; Ryan et al., 2003; Vítovec et al., 2006; Hamnes et al., 2007; Langkjaer et al., 2007; Suárez-Luengas et al., 2007). In the present study, *Cryptosporidium* oocysts were detected in the faeces of 21.1% of the samples (87/413).

Table 1
Prevalence of *Cryptosporidium* in pigs from three complexes on the farm in the Czech Republic diagnosed by microscopy and PCR.

Complex	Age category	No. tested	No. positive		Prevalence (microscopy)	*Cryptosporidium* genotyping		
			Microscopy	PCR		*C. suis*	PII	*C. muris*
Breeding complex 1	Pre-weaners	56	21	15	37.5	15	0	0
	Starters	58	22	21	37.9	19	1	1
	Sows	25	1	0	4	0	0	0
Breeding complex 2	Pre-weaners	63	5	3	7.9	3	0	0
	Starters	73	16	11	21.9	7	4	0
	Sows	15	0	0	0	0	0	0
Growing complex	Pre-growers	123	21	19	13.9	1	17	1

PII *Cryptosporidium* pig genotype II.