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Cryptosporidium and *Giardia* in Danish organic pig farms: Seasonal and age-related variation in prevalence, infection intensity and species/genotypes

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ABSTRACT

Although pigs are commonly infected with *Cryptosporidium* spp. and *Giardia duodenalis*, including potentially zoonotic species or genotypes, little is known about age-related infection levels, seasonal differences and genetic variation in naturally infected pigs raised in organic management systems. Therefore, the current study was conducted to assess seasonal and age-related variations in prevalence and infection intensity of *Cryptosporidium* and *Giardia*, evaluate zoonotic potential and uncover correlations between species/genotypes, infection intensity and faecal consistency. Shedding of oocysts and cysts ((oo-)cysts) was monitored at quarterly intervals (September 2011–June 2012) in piglets ($n = 152$), starter pigs ($n = 234$), fatteners ($n = 230$) and sows ($n = 240$) from three organic farms in Denmark. (oo-)Cysts were quantified by immunofluorescence microscopy; and 56/75 subsamples from *Cryptosporidium* infected pigs were successfully analysed by PCR amplification and partial sequencing of the small subunit (SSU) 18S rRNA and *hsp70*genes, while 13/67 *Giardia* subsamples were successfully analysed by amplification and partial sequencing of the 18S rRNA and the *gdh* genes. Altogether, *Cryptosporidium* or *Giardia* infections were observed in 40.9% (350/856) and 14.0% (120/856) of the pigs, respectively, including 8.2% (70/856) infected with both parasites. Prevalence, intensity of infections and presence of *Cryptosporidium* species varied significantly between age-groups; 53.3% piglets, 72.2% starter pigs, 40.4% fatteners and 2.9% sows were infected with *Cryptosporidium*, whereas 2.0% piglets, 27.4% starter pigs, 17.8% fatteners and 5.0% sows were infected with *Giardia.* The overall prevalence was stable throughout the year, except for dual-infections that were more prevalent in September and December ($p < 0.05$). The infection intensity was age-related for both parasites, and dual-infected pigs tended to excrete lower levels of oocysts compared to pigs harbouring only *Cryptosporidium*. Likewise, pigs infected with *Cryptosporidium scrofarum* excreted fewer oocysts (mean CPG: 54,848 ± 194,508CI: 9085–118,781) compared to pigs infected with *Cryptosporidium suis* (mean OPG: 351,035 ± 351,035CI: 67,953–634,117). No correlation between faecal consistency and (oo-)cyst excretion levels was observed.

Of the successfully genotyped isolates, 38/56 (67.9%) were *C. scrofarum* and 18/56 (32.1%) were *C. suis*, while the livestock specific *G. duodenalis* Assemblage E was detected in 11/13 (84.6%) isolates and the potentially zoonotic Assemblage A was identified in 2/13 (15.4%) isolates. Piglets exclusively hosted *C. suis,* with one exception, while starter pigs and fatteners predominantly hosted *C. scrofarum.* As organic pigs are partly reared outdoors, environmental contamination with *Cryptosporidium* and *Giardia* is inevitable. Nevertheless, the present data indicate that the potential public health risk associated with both of these parasites in Danish organic pig production seems to be negligible.

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1. Introduction

Protozoan parasites belonging to the genera *Cryptosporidium* and *Giardia* are prevalent in a variety of animal species, including cattle, pigs and humans (Current and Garcia, 1991; Maddox-Hyttel et al., 2006). Both parasites can cause severe symptoms, primarily diarrhoea, e.g. in young calves and humans. In addition, giardiasis is known to induce chronic malnutrition and growth retardation with subsequent risk of impaired cognitive function in humans (Berkman et al., 2002). Moreover, post-giardiasis complications such as fatigue and abdominal symptoms years after infection have been observed (Morch et al., 2009, 2013; Naess et al., 2012). Natural porcine infections with either of the parasites are typically asymptomatic (Quilez et al., 1996; Maddox-Hyttel et al., 2006; Kváč et al., 2009a; Nemejc et al., 2013; Zhang et al., 2013), whilst watery diarrhoea, anorexia and increased mortality have been described in piglets experimentally infected with *Cryptosporidium parvum* (Tzipori et al., 1982; Enemark et al., 2003).

Both parasites can be transmitted directly through contact with infected individuals or indirectly by consumption of faeces contaminated food or water (Meinhardt et al., 1996; Fayer, 2004). Livestock, in particular cattle, has been implicated as a major source of surface water contamination with *Cryptosporidium* and *Giardia* (oo-)cysts (Bodley-Tickell et al., 2002; Thurston-Enriquez et al., 2005). Moreover, *Cryptosporidium* contamination of water sources through surface run-off from faeces deposited on agricultural land has repeatedly been documented (Tate et al., 2000; Davies et al., 2004; Thurston-Enriquez et al., 2005). In addition, Petersen et al. (2012) demonstrated that *C. parvum* oocysts present in pig slurry applied to soil can leach and potentially contaminate groundwater. Cryptosporidiosis and giardiasis are widespread and debilitating diseases in calves, and cattle often host the zoonotic *C. parvum* and to a lesser degree also *Giardia duodenalis* Assemblage A. Thus, cattle have frequently been the subject of *Cryptosporidium* and *Giardia* surveys (Appelbee et al., 2003; Maddox-Hyttel et al., 2006; Silverlås and Blanco-Penedo, 2013). The opposite applies for pigs, where cryptosporidiosis and giardiasis generally have been disregarded. Additionally, pigs are mostly reported to host *Cryptosporidium suis* and *Cryptosporidium scrofarum* (Ryan et al., 2003; Langkjaer et al., 2007; Yin et al., 2013; Nemejc et al., 2013; Nguyen et al., 2013; Zhang et al., 2013), that are considered relatively host specific; and *G. duodenalis* Assemblage E (Langkjaer et al., 2007; Armson et al., 2009; Sprong et al., 2009), which is restricted to livestock. Recently, the awareness of porcine infections with these protozoan parasites has, nevertheless, increased worldwide. This has led to occasional observations of the zoonotic *C. parvum* and *G. duodenalis* Assemblage A in pigs (Xiao et al., 2006; Leoni et al., 2006; Langkjaer et al., 2007; Armson et al., 2009; Kváč et al., 2009b), as well as *C. andersoni* (Hsu et al., 2008), *C. muris* (Zintl et al., 2007), *C. tyzzeri* (Budu-Amoako et al., 2012), *G. duodenalis* Assemblage C, D, E and F (Langkjaer et al., 2007; Armson et al., 2009; Minetti et al., 2014), all of which have been documented sporadically in humans (Leoni et al., 2006; Sprong et al., 2009; Raskova et al., 2013). However, none of these studies provided a comprehensive description of seasonal and age-related differences in prevalence or genetic variation of *Cryptosporidium* and *Giardia* in outdoor reared pigs. Detailed knowledge of potential zoonotic transmission is furthermore hampered because only few studies in pigs have characterised specimens by sequence analysis of molecular markers (Sprong et al., 2009).

As pigs have the capability to excrete extremely high numbers of oocysts and cysts per gram of faeces (OPG and CPG) (Fayer et al., 2006; Maddox-Hyttel et al., 2006), fertilisation of agricultural land with slurry or faecal deposition directly on the ground from infected free-range pigs can eventually be a source of human waterborne cryptosporidiosis and giardiasis. Organic pigs, contrary to conventionally reared pigs, have access to outdoor areas throughout most of their lives. Therefore, this study was conducted to: (1) assess seasonal- and age-related variations in prevalence and infection intensity of *Cryptosporidium* and *Giardia* in organic pig farms, (2) to evaluate the zoonotic potential of these infections through DNA sequence analyses, and (3) to uncover any correlation between species/genotypes hosted by the pigs, infection intensity and faecal consistency in the pigs.

2. Material and methods

2.1. Study farms

Three commercial Danish organic pig farms with laboratory confirmed *Cryptosporidium* and *Giardia* infections were selected for the study based on the owner's willingness to participate. The farms were visited at quarterly intervals from September 2011 to June 2012. In all farms, piglets were born outdoor on farrowing pastures and weaned at seven to nine weeks of age. The farrowing pastures were subdivided into smaller paddocks, with one to five sows separated by a single electrical wire; in practice, allowing the piglets free access to the entire area. Post-weaning, the starter pigs were housed indoor in concrete pens with access to a partially roofed outdoor area. Moreover, all weaned pigs were provided with straw bedding up to 25 cm deep.

On farm 1, weaned pigs from several sows were randomly allocated into pens accommodating 20–25 pigs. At 35 kg (≈15 weeks of age) the group was split in two, and half of the pigs were relocated to a new pen. The stable was partly roofed with no clear distinction between indoor and outdoor areas. Bedding was deepest underneath the roofed part of the stable and gradually decreased towards the open part where the floor was partially slatted. On farm 2 and 3, starter pigs and fatteners were kept in separate, fully roofed units with separate outdoor runs and separate feeding areas.

2.2. Sample collection

Individual faecal samples were collected rectally. At each visit, we aimed at taking 20 samples from sows (10 pregnant and 10 lactating), fatteners (80–100 kg) and starter pigs (20–30 kg corresponding to 9–14 weeks of age), respectively. In addition, samples were collected immediately after defecation from 9 to 19 suckling piglets (≤7 weeks) in the farrowing paddocks. The samples were transported on ice to the laboratory and stored at 5 °C until processing.

2.3. Faecal examination

Faecal consistency was assessed on a scale from one to five (1: normal; 2: soft, 3: liquid; 4: watery; 5: watery with blood and/or intestinal tissue). *Giardia* cysts and *Cryptosporidium* oocysts ((oo-)cysts) were isolated and enumerated as previously described by Maddox-Hyttel et al. (2006). Briefly, 1 g faeces was suspended by vortexing in 3.5 mL 0.01% Tween 20 and filtered through multilayered 20-thread gauze (a 5 cm × 5 cm piece placed in a 10 mL syringe and held in place by a piece of wire). Another 3.5 mL 0.01% Tween 20 was added and the fluid pressed through the gauze with the syringe piston. The filtrate was under layered with 3.5 mL flotation fluid (saturated saline with glucose (50 g in 100 mL) diluted 1:1 with MQ water (final specific gravity = 1.13 g mL^{-1}) and centrifuged at 53 × *g* for 3 min. The supernatant was transferred to a clean tube and washed three times using MQ water and subsequently centrifuged at 1540 × *g* for 10 min to obtain a final sample volume of 2 mL. For each sample, a 10 μL subsample was placed in a well on a 3-well Teflon printed diagnostic slide (Immuno-Cell Int., Belgium)