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High prevalence of *Blastocystis* sp. in pigs reared under intensive growing systems: Frequency of ribotypes and associated risk factors

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Abstract

Three hundred and ninety-five pig fecal samples were analyzed looking for *Blastocystis* sp. using optical microscopy and PCR. A global prevalence of 46.8% has been observed in this study, although relative values of prevalence differ notably according to the strata examined, ranging from 9.3% in sows to 75% in weaners. Statistic analysis of the data included several risk factors such as different management systems, date of sample collection, fecal consistency, age and sex of the animals. The presence of the parasite was statistically associated to the variables ''age'' and ''date of sample collection'', being more prevalent in weaners and grower pigs and warm season. Random fragment-length polymorphism (RFLP-PCR) analysis of positive PCR samples revealed a high homology in the digestion pattern, appearing as two ribotypes. The results were further confirmed by sequencing of ten randomly selected samples, showing that the samples obtained in this study were included in two genotypes: genotype I previously named by Noël et al. [Noël, C., Dufernez, F., Gerbod, D., Edgcomb, V.P., Delgado-Viscogliosi, P., Ho, L.-Ch., Singh, M., Wintjens, R., Sogin, M.L., Capron, M., Pierce, R., Zenner, L., Viscogliosi, E., 2005. Molecular phylogenies of *Blastocystis* isolates from different hosts: implications for genetic diversity, identification of species, and zoonosis. J. Clin. Microbiol. 43, 348–355], in which *Blastocystis* sp. sequences from humans, pigs and cattle were included, and genotype II, which only included *Blastocystis hominis* sequences obtained from human and other primates. This is the first report including *Blastocystis* sequences from swine origin in genotype II.

Keywords: *Blastocystis*; Pig; Zoonosis; Risk factor

1. Introduction

Blastocystis is a protistan that infects a wide range of hosts, including humans and other mammals, and non-mammalian animals such as avian species, reptiles and insects (Stenzel and Boreham, 1996; Duda et al., 1998; Tan et al., 2002). Since no agreement has yet been

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reached regarding the speciation of this genus, *Blastocystis hominis* has been proposed for those isolated from humans, and *Blastocystis* sp. for those obtained from animals (Stenzel and Boreham, 1996; Yoshikawa et al., 2004a). Antigenic, karyotipic and genetic heterogeneity has also been demonstrated in various studies (Stenzel and Boreham, 1996; Carbajal et al., 1997; Arisue et al., 2003; Yoshikawa et al., 2003).

Blastocystis is considered by many authors to be a potential pathogen, because the number of forms found in feces of symptomatic human patients is relatively high (for review, see Zierdt, 1991; Stenzel and Boreham, 1996). It has been also proposed that *Blastocystis* could be responsible for allergic manifestations in human patients (Giacometti et al., 2003, among others). Its pathogenicity is a controversial point, since it also appears in asymptomatic individuals, although usually in much smaller numbers. This fact has been observed in many other intestinal pathogens such as *Giardia* or *Cryptosporidium*, where shedding of resistance forms in feces persisted for days or even months after the initial infection and resolution of clinical signs. The association of protozoa infection (including *Giardia intestinalis*, *Dientamoeba fragilis* and *B. hominis*) with irritable bowel syndrome (IBS) has been suggested by many authors, and a routine parasitological investigation of individuals with IBS is highly recommended by Stark et al. (2007). Moreover, in a recent study carried out by Dagci et al. (2007) a significant increase in the intestinal permeability has been found in individuals infected with *G. intestinalis* or *B. hominis* when compared with healthy controls. This fact was not observed in patients carrying only *Entamoeba coli*.

Blastocystis displays a broad genetic diversity, as mentioned by many authors (Stenzel and Boreham, 1996; Carbajal et al., 1997) and could comprise many different species, but this point is yet to be elucidated and more studies are needed, including different criteria such as DNA analysis of conserved regions, hosts of origin, requirements for cultivation or the presence of pathogenic factors, such as adhesins or other attachment molecules. Many different methods have been employed to analyze the genotype of *Blastocystis*, the most commonly used being ribotyping and sequencing of the small subunit ribosomal RNA (SSR rRNA) gene. After re-examining available sequences, Noël et al. (2003, 2005) and Stensvold et al. (2007b) reached the same conclusion: up to seven clearly differentiated genotypes are distinguished, including those described by Clark (1997) and Böhm-Gloning et al. (1997). These studies indicate that, although it appears to be a more limited approach, restriction-fragment-length polymorphism could be useful for genotype differentiation among *Blastocystis* positive samples.

In various studies, isolates of *Blastocystis* from different animal hosts have been analyzed looking for zoonotic genotypes. Pigs have been included among the hosts analyzed in separate studies and *Blastocystis* genotypes from pigs are grouped together with *Blastocystis* genotypes from humans in the same cladogram, showing the potential zoonotic of this protozoan (Abe et al., 2002, 2003; Noël et al., 2003, 2005; Abe, 2004). *Blastocystis* has been mentioned as a common parasite in pigs in several studies carried out in different parts of the world, including Spain (Burden et al., 1978/1979; Pakandl, 1991; Venturini et al., 1994; Quilez et al., 1995; Abe et al., 2002), but no broad studies in this animal species analyzing different management systems and determining the genotype have been conducted. In this study, we analyzed a statistically significant number of samples in order to determine the prevalence and role of *Blastocystis* in pig farms, including analysis of risk factors affecting the presence of this protistan and the main genotypes found in pigs.

2. Materials and methods

2.1. Design of the sampling

The minimum size of the sample (n) was determined in 384 samples, applying the formula to estimate a proportion "$n = Z\alpha^2\ [P(1-P)/L^2]$". For this purpose, the most disfavoured prevalence value ($P = 50\%$), a confidence level of 95% ($Z\alpha = 1.84$) and an assumed error (L) of 5% (Hsieh et al., 1998) was taken. A census of the total pig farms in the Valencian Community (1,129,055 animals) was used as reference for the composition of the pig population (MAP, 2002). Using a clustered sampling approach, individual faecal samples from 395 animals were finally collected from 11 pig farms, including 3 different management systems distributed as follows: 4 farrow-to-finish farms (FF), 2 growing-to-finish farms (GF) and 5 farrow-to-weaning farms (FW) (sows and piglets). These farms housed the animals indoors during all their life and cleaning was made in a routine manner between lots of animals. The age of the animals was considered the main stratum when sampling was designed. In the estimation published in December 2002 by the Ministry of Agriculture (MAP), a total of 422,349 animals less than 6 months (growers) and 123,874 reproductive