DETECTION OF INTESTINAL PARASITES IN PIG SLURRIES COLLECTED FROM FARMS IN THE ALICANTE PROVINCE (SPAIN)

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ABSTRACT

The aim of this study was to investigate the presence of intestinal parasites in pig slurries from several piggeries in Alicante (Spain). Pig slurries were collected in five high intensive pig farms (A-E) and sampled in each farm from the pits depending on the production cycle (gestating sows, farrowing sows, weaners, finishers). Samples were concentrated either through zinc sulphate flotation or by formalin-ethyl acetate sedimentation methods. Parasitological examination was performed by optical microscopy. Detection of *Cryptosporidium* sp. was performed using conventional acid-fast stain and by DNA extraction and PCR amplification. *Cryptosporidium* genus-specific primers (CPBDIAGF and CPBDIAGR) were used to amplify the *Cryptosporidium* SSU-rRNA variable region. Intestinal parasites were observed in all farms studied. Several protozoa (*Ballatidium coli*, *Entamoeba coli* and *Cryptosposidium* sp.) and helminths (*Ascaris suum*, *Trichuris suis, Fasciola hepatica*, Strongylida and nematode larvae) were identified. Parasite viability studies are needed in order to asses the potential concern on animal and human health.
Keywords: *parasites, pig slurries, protozoa, helminths.*

INTRODUCTION

The application of raw livestock wastes in agricultural soils is one of the most extended practices for residue management. However, there are diverse components in their composition, especially pathogens, heavy metals and salinity, which are potentially dangerous for the environment and for man. Swine faeces are a source of pathogenic organism, mainly bacteria, viruses, parasites and fungi. The most frequently found parasites in intensified hog farming are *Ascaris suum*, *Trichuris suis, Strongyla, Ballantidium coli* and *Cryptosporidium* spp. (Caballero-Hernández et al. 2004), some of these have been able to survive in the environment. Waterborne transmission of intestinal parasites has been linked to domestic livestock and farming practices. The danger for humans of becoming infected with protozoa with animal origin is higher than with helminths (Burton and Turner, 2003). *Cryptosporidium* sp. robust oocysts can survive for long periods outside the host, particularly in moist environments. Mawdsley et al. (1996) demonstrated that *Cryptosporidium* oocysts can move through various soil types, and Lindergard et al. (2001) concluded that in general, oocysts isolated from soil samples are regarded as being viable and potentially infective to humans. As an example, *Ascaris suum* eggs were not destroyed when the solid fraction of swine manure was ensiled for 56 days (Caballero-Hernández et al. 2004), therefore could be dangerous in the feeding of other animals. The aim of this study was to investigate the presence of intestinal parasites (protozoa and helminths) in pig slurries from several piggeries in Alicante (Spain).

MATERIAL AND METHODS

Pig slurries were collected in five high intensive pig farms (A-E) in Alicante Province

(Spain), and sampled in each farm from the pits depending on the production cycle (gestating sows, farrowing sows, weaners, finishers). In the farms A to D, the production cycle was complete and E had only the stages of farrowing sows and weaners. 25-l slurry samples were collected, after a mechanical homogenisation of the whole volume of the pit, when aged 30-60 days and immediately analysed in the laboratory. Samples were concentrated either through zinc sulphate flotation or by formalin-ethyl acetate sedimentation methods. Parasitological examination was performed by optical microscopy. Detection of *Cryptosporidium* sp. was carried out by Kinyoun carbol-fuchsin modified acid-fast stain (Melvin and Brooke, 1982) and direct immunofluorescence (Garcia et al. 1992). Stained slides were examined by observing 200 oil-immersion fields. DNA extraction and PCR amplification of the diagnostic region of the small subunit ribosomal RNA (SSUrRNA) gene using CPB-DIAGF and CPB-DIAGR primers (da Silva et al., 1999; Johnson et al., 1995)

RESULTS AND DISCUSSION

Intestinal parasites were observed in all farms studied. The distribution of the species (protozoa and helminths) is shown in table 1.

***Table 1**. Protozoa and helminths observed in the study.*

	Protozoa			
Farm	Gestating sows	Farrowing sows	Weaners	Finishers
A	*Ballatidium coli* *Cryptosporidium* sp.	*Ballatidium coli* *Cryptosporidium* sp.	*Ballatidium coli* *Cryptosporidium* sp.	*Ballatidium coli* *Cryptosporidium* sp.
B	*Ballatidium coli* *Entamoeba coli* *Cryptosporidium* sp.	*Ballatidium coli* *Entamoeba coli* *Cryptosporidium* sp.	*Ballatidium coli*	ND
C	*Ballatidium coli* *Entamoeba coli*	*Ballatidium coli* *Entamoeba coli*	*Entamoeba coli*	*Entamoeba coli*
D	ND	*Ballatidium coli* *Entamoeba coli*	*Ballatidium coli* *Entamoeba coli*	*Ballatidium coli*
E	No tested	*Ballatidium coli*	*Ballatidium coli*	No tested
	Helminths			
Farm	Gestating sows	Farrowing sows	Weaners	Finishers
A	nematode larvae	ND	ND	ND
B	*Ascaris suum* *Fasciola hepatica*	*Ascaris suum* *Fasciola hepatica* *Trichuris suis* Strongylida	Strongylida	ND
C	*Ascaris suum* nematode larvae	Strongylida	Strongylida	Strongylida
D	Strongylida nematode larvae	Strongylida	Strongylida	Strongylida
E	No tested	*Trichuris suis* Strongylida	Strongylida	No tested

¥ ND: no detected

Protozoa

Three main protozoa species were detected in the pig slurries, *Ballantidium coli*, *Entamoeba coli* and *Cryptosposidium* sp. *Ballantidium coli* was observed in the 78% of the pig slurries analysed, in all the farms, showing a non-specific affinity with any production stage, according to the role of pig as a principal reservoir of this protozoa. The prevalence of *Ballantidium* is fre-

quent and its resistance is not well known (Burton and Turner, 2003). In addition, *Ballantidium* could be an occasional human pathogen, specially related to farmers (Garcia, 1999). *Entamoeba coli* were detected in three of the farms, in the 44% of the pig slurries analysed.

Cryptosporidium sp. was detected by PCR in two farms (figure 1), observing a higher presence in the stages of gestating and farrowing sows, probably due to the immunosuppressant nature of these production stages of pigs, according to its opportunism nature of this parasite. This coccidian parasite was previously identified in pigs from other regions in Spain (Quilez et al., 1996). Awad-el-Kariem et al. (1998) concluded that not all isolated *Cryptosporidium parvum* excreted by animals are pathogenic to humans, but those adapted to humans always are. Recent reports show the presence of human infection by the pig genotype of *Cryptosporidium parvum* (Cama et al., 2003).

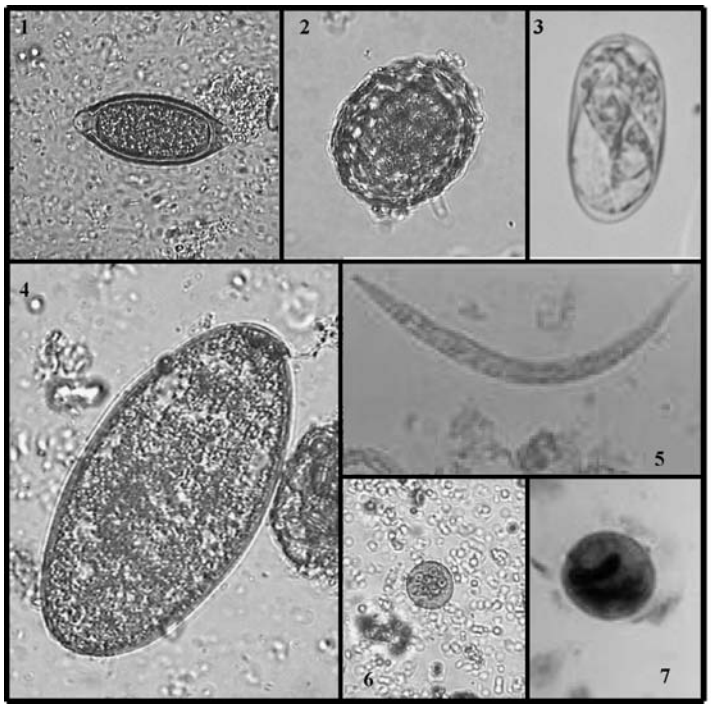

***Figure 1**. 1, Trichuris suis egg; 2, Ascaris suum egg, 3, Strongylida egg; 4, Fasciola hepatica egg; 5, nematode larvae; 6, Entamoeba coli cyst; 7, Ballatidium coli cyst.*

<u>Helminths</u>

In our study, no cestode eggs were detected in pig slurries. Nematode larvae were detected in three farms, and its presence was associated only to gestating sows. Strongylida eggs were detected, but a higher specific classification was impossible. Strongylida was the most prevalent helminth in the pig slurries (56% of the samples), and its was present in all the production stage, except in gestating sows. *Trichuris suis* and *Ascaris suum* eggs was detected in 11 and 17% of the samples respectively, always in slurries from gestating or farrowing sows. The morphology of the eggs was perfectly conserved, and probably the parasite remains viable as it has been reported in other studies (Gaasenbeek and Borgsteede, 1998). *Fasciola hepatica* was detected in farm A, only in the gestating and farrowing sows production stage, according to the heteroxen

cycle of this parasite. The input of this parasitation could be feeding or incoming of parasited sows.

Due to the extended application of raw livestock wastes in agricultural soils, viability studies are needed in order to assess the potential concern on animal and human health of the parasites present in these samples.

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