Short communication

Detection of intestinal parasites in pig slurry: A preliminary study from five farms in Spain☆


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Abstract

The aim of this study was to investigate the presence of intestinal parasites in pig slurries from several pigeries in Alicante (Spain). Pig slurries were collected in five highly-intensive pig farms (A–E), being 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 found in all farms studied. Several protozoa (Ballantidium coli, Entamoeba coli and Cryptosporidium sp.) and helminths (Ascaris suum, Trichuris suis, Fasciola hepatica, Strongylida and nematode larvae) were identified. Parasite viability studies are needed in order to assess the potential risk for animal and human health.

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Keywords: Parasites; Pig slurries; Protozoa; Helminths

1. Introduction

The application of livestock manure 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 salts, which are potentially dangerous for the environment and for man. Swine faeces are a source of pathogenic organisms, mainly bacteria, viruses, parasites and fungi. The most frequently found parasites in intensified hog farming are Ascaris suum, Trichuris suis, Strongylida, Ballantidium coli and Cryptosporidium spp. (Caballero-Hernández et al., 2004), some of which have been able to survive in the environment. Water-borne transmission of intestinal parasites is a serious health concern.
parasites has been linked to domestic livestock and farming practices. The danger for humans of becoming infected with protozoa of 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, A. suum eggs were not destroyed when the solid fraction of swine manure was ensiled for 56 days (Caballero-Hernández et al., 2004), so there could be a subsequent danger of infection through animal feed.

In the Spanish Community of Valencia State of Spain, the number of commercial swine herds is on the increase. The main type of management is completed cycle, which accounts for the 49% of total pig slurry production. 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).

2. Material and methods

Pig slurries were collected in five highly-intensive pig farms (A–E) in Alicante Province (Spain), samples were taken from the pits depending on the production cycle (gestating sows, farrowing sows, weaners, finishers). In farms A to D, the production cycle was complete ($x=200$) whilst E only had farrowing sows ($n=6000$) and weaners ($n=1500$). All the farms practiced intensive management, mainly with dry-feeding using pellets and/or flour. The livestock manure was collected in separated pits depending on the production stage. Cleaning systems usually included high pressure water.

Slurry samples (25 l) were collected, after 30–60 days, following a mechanical homogenisation of the whole pit volume. Samples were preserved with formalin 10% ($v/v$) and with absolute ethanol ($v/v$) and maintained at 4°C. One-hundred millilitres of formalin fixed samples were sieved, washed with distilled water and allowed to sediment for 24h. At this point, the supernatant was decanted and 10 ml of the sediment was concentrated by formalin–ethyl acetate sedimentation method (Ash and Oriehl, 1991). The remaining sediment was concentrated by acetate–acetic–ether sedimentation followed by zinc sulphate flotation (density 1.18). All samples were processed in triplicate. Parasitological examination was performed by optical microscopy. Detection of Cryptosporidium sp. was carried out by Kinyoun carbol-fuchsin modified acid-fast staining (Melvin and Brooke, 1982) and direct immunofluorescence (Garcia et al., 1992). Stained slides were examined by observing 200 oil-immersion fields. In addition, an aliquot of approximately 300μl of each pig slurry preserved in ethanol was suspended in 1 ml of 0.01M phosphate-buffered saline, pH 7.2, containing 0.01 M EDTA (PBS-EDTA), and the suspension was centrifuged at 14,000×g for 5 min, at 4°C. The pellet from this centrifugation was washed two more times under the same conditions. The pellet was re-suspended in 300μl of PBS-EDTA and used for DNA extraction, performed with the FastPrep disrupter and the FastDNA kit (BIO 101, Inc., Vista, CA) (da Silva et al., 1999). Extracted DNA was stored at 4°C until PCR amplification.

Cryptosporidium genus-specific primers (CPBDIAGF and CPBDIAGR) were used to amplify the Cryptosporidium SSU-rRNA variable region (Johnson et al., 1995). The conditions for PCR were 95°C for 15 min; 45 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s, extension at 72°C for 90 s; followed by extension at 72°C for 9 min; finishing with a hold step at 4°C.

PCR products were analysed by electrophoresis on 2% SeaKem GTG agarose (Cat. No. 50074, FMC Bioproducts, Rockland, ME), stained with ethidium bromide, and visualised on a UV transilluminator.

3. Results and discussion

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

3.1. Protozoa

Three main protozoa species were detected in the pig slurries: B. coli, Entamoeba coli and Cryptosporidium sp. Giardia spp. cysts and Eimeria spp. or Isospora spp. oocysts were not observed. B. coli cysts
(45–65 μm, Fig. 2G) were observed in 78% of the pig slurries analysed from all the farms, showing no specific affinity with any production stage, in accordance with the role of pigs as a principal reservoir of this protozoa. The prevalence of *B. coli* is common and its resistance is not well known (Burton and Turner, 2003). Hindsbo et al. (2000) reported that the prevalence of *B. coli* infection increased from 57% in suckling piglets to 100% in most pig groups more than 4 weeks old. In addition, *B. coli* can be an occasional human pathogen, especially for farmers (Garcia, 1999).

*E. coli* cysts (10–30 μm, Fig. 2F) were detected in three of the farms, totaling 44% of the pig slurries analysed.

*Cryptosporidium* sp. was detected in farms A and B. Stained oocysts were observed by optical microscopy in the stages of gestating and farrowing sows. PCR analysis also revealed the presence of the parasite in the weaner and finisher stages in farm A. (Fig. 1). This coccidian parasite was previously identified in pigs from other regions of Spain (Quilez et al., 1996). Quilez et al. (1996) identified *Cryptosporidium parvum* oocysts in 136 (21.9%) of pigs from 21 (77.8%) farms in the region of Aragón.

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**Table 1**

Protozoa and helminths observed in the study

<table>
<thead>
<tr>
<th>Farm</th>
<th>Gestating sows</th>
<th>Farrowing sows</th>
<th>Weaners</th>
<th>Finishers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protozoa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td><em>Ballantidium coli</em></td>
<td><em>Ballantidium coli</em></td>
<td><em>Ballantidium coli</em></td>
<td><em>Ballantidium coli</em></td>
</tr>
<tr>
<td></td>
<td><em>Cryptosporidium</em> sp.</td>
<td><em>Cryptosporidium</em> sp.</td>
<td><em>Cryptosporidium</em> sp.</td>
<td><em>Cryptosporidium</em> sp.</td>
</tr>
<tr>
<td>B</td>
<td><em>Ballantidium coli</em></td>
<td><em>Ballantidium coli</em></td>
<td><em>Ballantidium coli</em></td>
<td>NDa</td>
</tr>
<tr>
<td></td>
<td><em>Entamoeba coli</em></td>
<td><em>Entamoeba coli</em></td>
<td><em>Entamoeba coli</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Cryptosporidium</em> sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td><em>Ballantidium coli</em></td>
<td><em>Ballantidium coli</em></td>
<td><em>Entamoeba coli</em></td>
<td><em>Entamoeba coli</em></td>
</tr>
<tr>
<td></td>
<td><em>Entamoeba coli</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>ND</td>
<td><em>Ballantidium colix</em></td>
<td><em>Ballantidium coli</em></td>
<td><em>Ballantidium coli</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Entamoeba coli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Not tested</td>
<td><em>Ballantidium coli</em></td>
<td><em>Ballantidium coli</em></td>
<td>Not tested</td>
</tr>
</tbody>
</table>

| **Helminths** | | | |
| A | Nematode larvae | NDa | ND |
| B | *Ascaris suum* | *Ascaris suum* | Strongylida |
| | *Fasciola hepatica* | *Fasciola hepatica* Strongylida |
| C | *Ascaris suum* Nematode larvae | Strongylida |
| | | Strongylida |
| D | Strongylida Nematode larvae | Strongylida |
| | | Strongylida |
| E | Not tested | *Trichuris suis* Strongylida |
| | | Strongylida |

* ND: not detected.
Infection rates were significantly higher in weaned, 1- to 2-month-old piglets (59.2%) than in fattened, 2- to 6-month-old pigs (34.3%). Awad-El-Kariem et al. (1998) concluded that not all isolated *C. parvum* excreted by animals are pathogenic to humans, but those adapted to humans always are. Recent reports demonstrate human infection by the pig genotype of *C. parvum* (Cama et al., 2003).

### 3.2. Helminths

In our study, no cestode eggs were detected in pig slurries. We named as Strongylida eggs (Fig. 2C), those ranging from 42.5–57.5 × 20–32.5 µm, according to the procedure of Bernabeu et al. (2002), although morphometric differentiation between Rhabditoida type and Ancylostomatoidea type eggs remains difficult. Strongylida was the most prevalent helminth in the pig slurries (56% of the samples), and it was present in all production stages, except gestating sows. Nematode larvae were detected in three farms (Table 1), and their presence was associated only with gestating sows, but we could not discard the occurrence of free-living nematodes on the samples studied. *T. suis* (47.5–67.5 × 25–30 µm, Fig. 2A), and *A. suum* (55–75 × 35–50 µm, Fig. 2B).

![Fig. 2. (A) *T. suis* egg; (B) *A. suum* egg; (C) Strongylida egg; (D) *F. hepatica* egg; (E) nematode larvae; (F) *E. coli* cyst; (G) *B. coli* cyst (bar: 20µm).](image-url)
eggs were detected in 11% and 17% of the samples, respectively, always in slurries from gestating or farrowing sows. Joachim et al. (2001) reported similar results: *A. suum* eggs in 10.5% of the studied samples from piggeries of North-Western Germany. The morphology of the eggs was perfectly conserved, and probably the parasite remains viable as has been previously reported (Gaasenbeek and Borgsteede, 1998). Hardly any *Fasciola hepatica* eggs (120–150 × 63–90 µm, Fig. 2D) were observed in farm A, only in the gestating and farrowing sows production stages, in accordance with the heteroxen cycle of this parasite. The source of this infestation could be through feeding or the entry of parasitized sows. Swine fascioliasis has been reported in pigs from other geographical areas such as Africa (El-Rafaie et al., 1984), Asia (Boes et al., 2000) and South America (Mas-Coma et al., 1997), as well as, in European wild boar (Shimalov and Shimalov, 2000).

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

**Acknowledgements**

This research work was supported by the Research and Technology Office (OCYT) of the Valencia Government (Project Number GV00-007-16). The authors wish to thank Dr. David Walker for the English revision.

**References**


