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Livestock Science 102 (2006) 237–242

LIVESTOCK SCIENCE

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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 piggeries 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 *Cryptosposidium* 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.

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*, *Strongyla*, *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

☆ This paper is part of the special issue entitled "Biosecurity of Livestock Effluents", Guest Edited by Dr. José Martinez and Dr. François Madec.

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doi:10.1016/j.livsci.2006.03.023

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 swineherds 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% (3:1) 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 24 h. 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 300 µl of each pig slurry preserved in ethanol was suspended in 1 ml of 0.01 M 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 300 µl 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