Assessing the biosafety risks of pig manure for use as a feedstock for composting

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HIGHLIGHTS

- Biosafety risks of pig manure for use as a feedstock for composting were examined.
- *Salmonella* was detected in manure from 50% of pig farms investigated.
- *E. coli* counts were higher in manure from farms with high *Salmonella* seroprevalence.
- Manure separation may help to reduce pathogen counts prior to composting.
- Findings should be considered when selecting manure as a feedstock for composting.

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ABSTRACT

The objective was to assess the biosafety risks of pig manure for use as a feedstock for composting. *Salmonella* was detected in the manure from half of the 30 pig farms sampled, with 52% of isolates recovered identified as multi-drug resistant *S.* Typhimurium. The highest prevalence (60%) was found on *Salmonella* category 2 and 3 farms i.e. those with medium and high *Salmonella* seroprevalence, respectively, although this was not statistically significant. *Escherichia coli* counts were, however, significantly higher in manure from *Salmonella* category 3 farms. Manure separation may be useful as a means of reducing/eliminating pathogens from manure prior to composting, as manure solids generated using a decanter centrifuge had lower *E. coli* and *Enterococcus* counts than manure. These findings should be taken into consideration when selecting pig manure for use as a feedstock for compost or other marketable manure by-products.

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1. Introduction

Composting of the separated solid fraction of pig manure is technically feasible, producing compost with acceptable physico-chemical properties (Nolan et al., 2011; Troy et al., 2012). However, biosafety is also a concern, considering that pig manure commonly harbors pathogens, most notably *Salmonella* (Létourneau et al., 2010) which may be carried over to the end-product. For this reason, marketable pig manure-derived compost, being a processed manure product, must comply with microbiological criteria as set out in the EU animal by-product regulations [Commission Regulation (EU) No 142/2011; EC, 2011]. This requires absence of *Salmonella* in representative 25 g samples and reduced counts of fecal indicator bacteria. Similar criteria are also set out in compost standards in different countries (Hogg et al., 2002; NSAI, 2011).

It has previously been demonstrated that enteric pathogens are reduced or eliminated during composting of the solid fraction of pig manure, with the final product meeting microbiological criteria (McCarthy et al., 2011). However, in this and other studies (Rao et al., 2007; Ros et al., 2006) the manure feedstock usually comes from only one source. The origin of the manure may well influence microbiological parameters. For example, categorization of the herd with respect to *Salmonella* seroprevalence is liable to influence the *Salmonella* status of the manure (Funk and Gebreyes, 2004). Counts of fecal indicator bacteria are also likely to vary from one farm to another.

To date, a limited number of studies have screened manure or fecal samples from Irish pig herds for enteric pathogens with a view to assessing the risk of pathogen transmission to humans and other livestock (EFSA, 2009; Rowe et al., 2003; Watabe et al., 2003). However, detailed characterization of the pathogens recovered (i.e. molecular typing and antibiotic resistance profiling) is lacking. In addition, to the authors' knowledge, there are no data on counts of

fecal indicator bacteria in pig manure sampled from different farms. Furthermore, it is usually the separated solid fraction of manure that is composted and there is little information on the effect of manure separation on enteric pathogen counts. For example, pathogens may partition into either the liquid or solid fractions, depending on the separation method used. Lastly, although mesophilic anaerobic digestion of pig manure is practiced on-farm, there are little data available on its effect on microbial pathogens (Sobsey et al., 2001).

Therefore, with a view to using pig manure or its separated solid fraction as a feedstock for composting, the aims of this study were; (1) to determine if serological status of a pig herd is an accurate predictor of the presence of *Salmonella* in pig manure and (2) to obtain baseline counts of indicator micro-organisms from manure sampled from different pig herds categorized based on their *Salmonella* seroprevalence as well as the separated solid and liquid fractions of pig manure and anaerobically digested (AD) pig manure from selected farms.

2. Materials and methods

2.1. Pig manure sampling

Manure samples were collected on one occasion from the finisher houses of 30 commercial pig farms in Ireland between January 2009 and March 2010. Farms were chosen based on their categorization within the initial (pre-2010) Irish Pig *Salmonella* Control Programme. It should therefore be noted that any reference to farm categories in this study refers to this historical categorization system where farms with <10% *Salmonella* seroprevalence were classed as category 1, those with 11–49% seroprevalence as category 2 and those with >50% seroprevalence as category 3. Ten farms were sampled from each of these three categories. The feed form (meal or pellets) and type of delivery (wet or dry) on each farm were noted. At the time of sample collection 19 farms were feeding meal, eight were using pelleted feed, and the remaining three farms were using a combination of both meal and pellets. Of the 27 farms using only one form of feed, 10 were using wet delivery systems and 17 dry delivery systems. Manure samples (~100 ml) were obtained from manure storage tanks situated directly underneath finisher houses at a depth of 1 m below the crust or from a sluice, if present. They were collected into sterile containers and transported on ice to the laboratory where they were stored at 4 °C until analysis (within 24 h).

2.2. Pig manure separation and sampling of the solid and liquid fractions

In addition to the 30 pig manure samples outlined above, manure and AD pig manure and the separated solid and liquid fractions of both were also collected from two selected farms to examine the effect of manure separation on enteric pathogens. The first farm sampled was one of the category 1 farms surveyed as outlined in Section 2.1. On this farm, samples of manure from the farm storage tank as well as separated solid and liquid fractions of the manure were collected on three occasions over a five month period. The manure was separated using a decanter centrifuge (GEA Westfalia Separator UCD 205, GEA WestfaliaSurge GmbH, Bönen, Germany) with the aid of a coagulant and a flocculent as outlined by Nolan et al. (2011). The decanter centrifuge consisted of a horizontal cylinder continuously turned at high speed. Centrifugal force separates the liquid and solid fractions of the manure inside the cylinder, with both discharged separately. The second farm was not sampled as part of the manure survey outlined in Section 2.1 but was selected as it was anaerobically digesting pig manure. All samples were taken on two occasions (13 months apart) from this farm which was in category 2 on the first occasion and category 1 on the second. Pig manure was sampled from the farm storage tank, AD pig manure was sampled from the batch mesophilic anaerobic digester after 30 days of

digestion and the separated solid and liquid fractions of AD manure were also collected. Manure separation on this farm was by means of a rotary belt press separator (SCS Biotechnology, Oxford, UK). This type of separator uses mechanical pressure (as opposed to centrifugal force in the decanter separator) to separate manure solids from the liquid fraction. It consisted of a flat belt running between rollers. The liquid fraction is forced through the belt by the rollers and the solids are carried along on the belt and dropped into a collection unit. Additional information on both types of separators used in the present study can be obtained from Burton (2007). All samples were collected into sterile containers and transported on ice to the laboratory where they were stored at 4 °C until analysis (within 24 h).

2.3. Microbiological analysis of manure samples

2.3.1. Counts of indicator bacteria, yeasts and molds and aerobic spore-forming bacteria

Endogenous indicator bacteria (coliform, *Escherichia coli* and *Enterococcus*), yeasts and molds and aerobic spore-forming bacteria were enumerated in the manure and the separated solid and liquid fractions as outlined by Mc Carthy et al. (2011). Briefly, 25 g samples were homogenized in 225 ml of buffered peptone water (followed by heating to 80 °C for 10 min for sporeformers) and appropriate dilutions were pour-plated in duplicate onto a range of selective media. To enumerate aerobic spore-forming bacteria, 5 ml of the initial 1 in 10 dilutions of each of the manure samples was heated to 80 °C for 10 min, cooled on ice and serially diluted 10-fold in maximum recovery diluent. Relevant dilutions were pour-plated in duplicate on nutrient agar and the plates were incubated at 37 °C for 48 h. All microbiological media were obtained from Merck (Darmstadt, Germany).

2.3.2. Salmonella isolation and characterization

The presence/absence of *Salmonella* in 25 g samples of manure and its separated solid fraction and in 25 ml samples of the separated liquid fraction was determined according to standard procedures (ISO 6579:2002) with modified brilliant green agar (Merck) used for additional selective plating. Isolation and characterization of any *Salmonella* isolates recovered were performed as described by Mc Carthy et al. (2011). Two *Salmonella* isolates per sample were serotyped based on O- and H-group antigens according to the White Kaufmann Leminor scheme (Grimont and Weill, 2007) except for one farm, from which only one isolate was obtained. Antimicrobial susceptibility testing against a panel of 13 antimicrobials was performed according to the broth dilution method of the Clinical and Laboratory Standards Institute (formerly NCCLS) (NCCLS, 2008). The list of antimicrobial agents and the concentration ranges tested were as follows; ampicillin (0.5–32 μg/ml), cefotaxime (0.06–4 μg/ml), ceftazidime (0.25–16 μg/ml), chloramphenicol (2–64 μg/ml), ciprofloxacin (0.008–8 μg/ml), florfenicol (2–64 μg/ml), gentamicin (0.25–32 μg/ml) kanamycin (4–128 μg/ml), nalidixic acid (4–64 μg/ml), streptomycin (2–128 μg/ml), sulfamethoxazole (8–1024 μg/ml), tetracycline (1–64 μg/ml) and trimethoprim (0.5–32 μg/ml). *Salmonella* Typhimurium isolates were phage typed by the National *Salmonella* Reference Laboratory at Galway University Hospital, Ireland. Molecular typing of *Salmonella* isolates was performed by pulsed field gel electrophoresis (PFGE) using XbaI (New England Biolabs, Hitchin, Herts, UK) according to a standardized PulseNet protocol (CDC, 2002). Gel images were visualized and analyzed and dendrograms constructed as outlined by Mc Carthy et al. (2011).

2.4. Statistical analysis

Statistical analysis of the data was performed using the PROC mixed procedure of SAS (SAS, Cary, NC, USA). The variables of interest were coliform, *E. coli*, *Enterococcus*, yeast and mold and aerobic