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Utility of specific biomarkers to assess safety of swine manure for biofertilizing purposes

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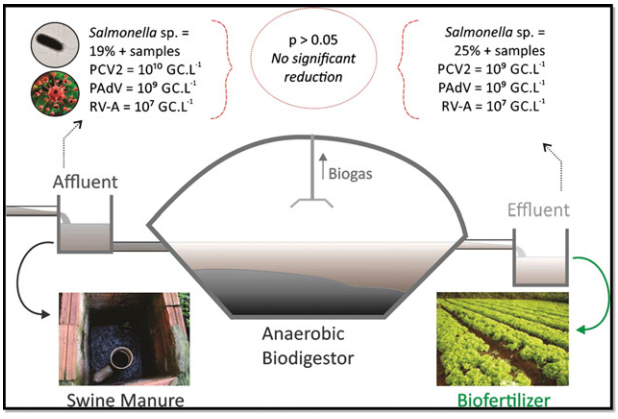
HIGHLIGHTS

- Enteric viruses and Salmonella spp. persist even after the anaerobic biodigestion of liquid swine manure
- PCV2, PAdV and RVA genomes were positive in 77.5%, 60% and 37.5%, of the samples respectively
- Salmonella spp. was found in 40% of the samples collected during the summer and in 15% during the winter
- It is necessary to establish more efficient sanitization methods for biofertilizer purposes from swine manure

GRAPHICAL ABSTRACT



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ABSTRACT

Swine production is an important economic activity in Brazil, and there is interest in the development of clean production mechanisms to support sustainable agro-industrial activities. The biomass derived from swine manure has good potential to be used as a biofertilizer due to its high nutrient concentration. However, the land application of manure should be based on safety parameters such as the presence of pathogens that can potentially infect animals and people. This study was designed to assess the presence of porcine circovirus-2 (PCV2), porcine adenovirus (PAdV), rotavirus-A (RV-A) and *Salmonella* spp. in liquid manure, as well the infectivity of two genotypes of circovirus-2 (PCV2a and PCV2b) present in liquid manure. Three swine farms were evaluated: 1) a nursery production farm (manure analyzed before and after anaerobic biodigestion), 2) a grow–finish production farm (analyzed before and after anaerobic biodigestion), and 3) a second grow–finish production farm (raw manure–affluent). PCV2, PAdV and RV-A were present before and after anaerobic biodigestion (either affluent or effluent) at all farms. *Salmonella* spp. were detected at farm 1 (affluent and effluent) and farm 3 (raw manure–affluent) but not farm 2 (affluent and effluent). When the ability of the anaerobic biodigestion process to reduce viral concentration was evaluated, no significant reduction was observed ($P > 0.05$). Both the PCV2a and PCV2b genotypes were detected, suggesting viral co-infection in swine production. The results revealed infectious PCV2 even after anaerobic biodigestion treatment. The presence of *Salmonella* spp. and enteric viruses, especially infectious PCV2, in the final effluent from the anaerobic biodigester system suggests that the process is inefficient for pathogen inactivation. Due to the prevalence and infectivity of PCV2 and considering the successful use of molecular methods coupled to cell culture for detecting

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infectious PCV2, we suggest that this virus can be used as a bioindicator in swine manure treatment systems to check the efficiency of pathogen inactivation and ensure the production of safe biofertilizers from swine manure.

1. Introduction

Concern with the development of clean mechanisms has encouraged Brazil to support research into sustainable agro-industrial activities. The use of anaerobic biodigesters for swine manure management allows effluent storage and biogas recovery, as well as the reuse of the final effluents as biofertilizers (Sobsey et al., 2006; Topp et al., 2009). This reuse is both ecologically and economically sound because the biomass derived from swine manure has nutritional potential to be used as a biofertilizer (Topp et al., 2009). However, safe reuse depends directly on safety parameters such as the presence of pathogens involved in diseases. Swine manure is characterized by high levels of microbial populations, including pathogenic bacteria such as *Salmonella* spp. and viruses such as adenovirus (PAdV), circovirus type 2 (PCV2) and rotavirus A (RV-A) (Hundesa et al., 2009; Shangjin et al., 2009; Viancelli et al., 2011).

Salmonella spp. is rod-shaped gram-negative bacteria that colonize the intestinal tract of animals and humans and are zoonotic pathogens (Griffith et al., 2006). PAdV, PCV2 and RV-A are non-enveloped viruses that are widespread within swine populations and are excreted in swine feces in high concentrations (Hundesa et al., 2009; Shangjin et al., 2009). PCV2 is the main infectious agent of post-weaning multisystemic wasting syndrome (PMWS), causing high mortality; porcine dermatitis and nephropathy syndrome (PDNS); enteric signs including diarrhea; and reproductive disorders. PCV2 is the cause of significant economic losses to the swine industry worldwide (Opriessnig et al., 2008). PCV2 is divided into the PCV2a, PCV2b and PCV2c genotypes (Segalés et al., 2008; Opriessnig et al., 2008). PCV2a predominates on pig farms with and without PMWS, while PCV2b is more commonly associated with outbreaks of PMWS (D. Kim et al., 2011; J. Kim et al., 2011). Three PCV2c sequences were identified in samples from Denmark during the 1980s when PMWS was not present (Dupont et al., 2008). RV-A is a major pathogen associated with acute gastroenteritis in animals and humans (zoonotic), and the disease is usually seen in young animals (Estes and Kapikian, 2007). PAdV is prevalent within swine populations and is found in feces, residual water, and sludge. Although PAdV does not produce clinically severe disease, it has been proposed as a viral bioindicator in wastewater treatment systems used in swine management and production (Maluquer de Motes et al., 2004; Hundesa et al., 2006; Viancelli et al., 2012).

Zoonotic pathogens (bacteria and viruses) can be present in biofertilizers of swine origin, which could pose a potential risk to the health of humans, animals, and the environment (Sobsey et al., 2006; Topp et al., 2009).

This study was designed to assess the presence and persistence of PAdV, PCV2, RV-A, and *Salmonella* spp. in liquid swine manure effluents collected at different farms and from different steps of the treatment process: raw manure and before (affluent) and after (effluent) passage through anaerobic biodigesters.

2. Materials and methods

2.1. Experimental design and manure sampling

In Brazil, the typical swine production farms are specialized in creating or nursery or grow–finish and some of the farms have both productions. Also in Brazil, many swine farms do not have an anaerobic biodigestion system for treating swine manure but, even though, they use the untreated wastes as biofertilizers in the field. The swine farms selected for the present study were well administrated and the animals had a general healthy routine life with occasional but not frequent outbreaks of diseases such as piglet diarrhea and/or PMWS due to PCV infections. They were located in Concórdia City, Santa Catarina State, Brazil (27°18′ S, 51°59′ W), a very traditional area that concentrates 64% of the swine production in Brazil and where the main swine industries are located as well. Three swine farms were evaluated for enteric viruses and *Salmonella* spp. in liquid swine manure: farm 1 (nursery production — approximately 400 animals); farm 2 (grow–finish production — approximately 300 animals), where the manure was collected before and after anaerobic biodigestion (hydraulic retention time of 30–40 days); and farm 3 (with grow–finish production — approximately 800 animals), without the swine treatment procedures (only tank waste storage) and the samples were collected raw (raw manure–affluent).

Fig. 1 shows the schematic representation of the anaerobic biodigester system (semicontinuous system) and the analyzed samples. As it is a semicontinuous biodigestion system, the samples collected at the entry of the system are not the same with those collected at the end of the system. To better represent the system dynamics, samples were collected weekly.

Forty samples from these farms were harvested weekly during the summer season of 2013 (January) (total of 4 sampling campaigns) and during the winter season of 2013 (July) (total of 4 sampling campaigns). Each sample was composed of 1 L of effluent, which was collected in suitable containers and immediately processed as described in Sections 2.2 and 2.3.

2.2. Physicochemical analyses

The sample temperatures (S. Temp.) and the environmental temperatures (E. Temp.) were measured immediately after collection. The sample total ammoniacal nitrogen (TAN), measured as NH_3–N, and the pH were measured according to APHA (2012).

2.3. Microbiological analyses

The qualitative analysis of *Salmonella* spp. was performed according to ISO 6579 (2002) and adapted for Michael et al. (2003). Briefly, 25 mL of sample was added to 225 mL buffered peptone–NaCl solution and incubated at 37 °C for 24 h. The solution was then added to Rappaport-Vassiliadis broth and tetrathionate and incubated at 42 °C for 24 h, followed by plating on brilliant green agar (BG) and in xylose-lysine-tergitol-4 (XLT4).

For viral analysis, 20 mL of samples were collected at each site. Samples were concentrated and submitted to DNA extraction as previously described by Viancelli et al. (2011). Briefly, 25 mL of sample was clarified and concentrated using the glycine buffer method coupled with polyethylene glycol precipitation. The viral particles were eluted from the precipitated sample using glycine buffer (pH 9.5) and further concentrated by PEG 6000 precipitation. After centrifugation, the supernatant was discarded, and the resulting pellet was suspended in 5.0 mL of 0.1 M phosphate buffer (pH 7.2).

As positive controls for viral recovery assays, swine manure samples and ultrapure water that had tested negative for RVA were inoculated with 8.0×106 GC mL^{-1} (genome copies per milliliter) of the Simian Rotavirus — SA11 and concentrated–clarified following the glycine–polyethylene glycol method as described in Section 2.3. The Simian Rotavirus — SA11 (group A, serotype G3) was propagated in MA104 cells (a continuous cell line derived from fetal rhesus kidney) and