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Survival of *Salmonella* spp. and fecal indicator bacteria in Vietnamese biogas digesters receiving pig slurry

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ABSTRACT

Small-scale biogas digesters are widely promoted worldwide as a sustainable technology to manage livestock manure. In Vietnam, pig slurry is commonly applied to biogas digesters for production of gas for electricity and cooking with the effluent being used to fertilize field crops, vegetables and fish ponds. Slurry may contain a variety of zoonotic pathogens, e.g. *Salmonella* spp., which are able to cause disease in humans either through direct contact with slurry or by fecal contamination of water and foods. The objective of this study was to evaluate the survival of *Salmonella* spp. and the fecal indicator bacteria, enterococci, *E. coli*, and spores of *Clostridium perfringens* in biogas digesters operated by small-scale Vietnamese pig farmers. The serovar and antimicrobial susceptibility of the *Salmonella* spp. isolated were also established. The study was conducted in 12 farms (6 farms with and 6 farms without toilet connected) located in Hanam province, Vietnam. Sampling of pig slurry and biogas effluent was done during two seasons. Results showed that the concentration of enterococci, *E. coli*, and *Clostridium perfringens* spores was overall reduced by only 1–2 $\log_{10}$-units in the biogas digesters when comparing raw slurry and biogas effluent. *Salmonella* spp. was found in both raw slurry and biogas effluent. A total of 19 *Salmonella* serovars were identified, with the main serovars being *Salmonella* Typhimurium (55/138), *Salmonella enterica* serovar 4,[5],12:i:- (19/138), *Salmonella* Weltevreden (9/138) and *Salmonella* Rissen (9/138). The *Salmonella* serovars showed similar antimicrobial resistance patterns to those previously reported from Vietnam. When promoting biogas, farmers should be made aware that effluent should only be used as fertilizer for crops not consumed raw and that indiscriminate discharge of effluent are likely to contaminate water recipients, e.g. drinking water sources, with pathogens. Relevant authorities should promote safe animal manure management practices to farmers and regulations be updated to ensure food safety and public health.

Introduction

Vietnamese pig production is rapidly increasing due to a human population and economy that are growing resulting in increased consumer demands for pork meat. In Vietnam, the per capita consumption of pork is expected to increase from 37.8 kg in 2012 to 48.7 kg by 2020 and pig production is projected to increase from 31.2 million pigs in 2012 to 34.8 million in 2020 (Stuart, 2012). In consequence, Vietnam is facing huge challenges managing large volumes of pig manure in an environmental sustainable manner (Huynh et al., 2006). Through surface run-off and seepage nitrate and other nutrients originating from manure may contaminate water sources including aquifers and wells used for extracting drinking water (Burton and Turner, 2003). Pig and other livestock manure may also contain a range of different zoonotic pathogenic bacteria, e.g. *Salmonella* spp., *E. coli*, *Campylobacter* spp., *Yersinia* spp.; parasites, e.g. *Ascaris* spp., *Taenia* spp. and protozoan parasites; and vira, e.g. Hepatitis E and exotic viruses like Nipah, that can cause disease in humans through direct exposure or comsumption of contaminated water or food (Guan and Holley, 2003; Herremans et al., 2007; Spencer and Guan, 2004). Pig manure is commonly used as crop fertilizer and *Salmonella* spp. and other pathogens frequently found in pig wastes (Hutchison et al., 2005a) may then contaminate the environment and crops (Baloda et al., 2001).

Vietnamese farmers have different ways to manage manure with most small-scale pig farms composting manure in heaps or

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digesting it in biogas systems. Solid pig manure is normally mixed with straw, ash or lime, then covered by mud or plastic and stored for a few months before use as fertilizer (Tuan et al., 2006; Vu et al., 2007). In farms integrating pig and fish production, both solid and liquid manure is discharged directly into fish ponds to fertilize growth of plankton and other organisms that is eaten by the fish (Vu et al., 2007). Large-scale farms store slurry mainly in lagoons but may also operate biogas digesters, or occasionally discharge the slurry into aquatic recipients.

Biogas digestion is an anaerobic process that generates little heat. The digestion is typically done at mesophilic (35–40 °C) or at thermophilic temperatures (50–55 °C) when systems are heated. Mesophilic digestion is associated with limited pathogen reduction which is in contrast to the thermophilic conditions where most pathogens are inactivated (Sari et al., 2011; Teodorita et al., 2008). In Vietnam, the use of biogas digesters to manage and treat livestock manure has grown fast since 2003 where main efforts were initiated to promote the technology (Khai and Luong, 2010). By the end of 2012, a total of 125,000 biogas units were built in Vietnam with support from the government as well as non-governmental organizations. Several designs of biogas digesters, e.g. fixed dome and plastic tube biogas digesters, have been developed addressing the needs of different livestock farming systems with the vast majority of biogas systems being small-scale digesters receiving pig manure from up to 20 fattening pigs (Biogas Program, 2012; Khai and Luong, 2010).

Biogas digestion of livestock manure provides renewable energy, potent fertilizer and a potential reduction in pollution of the environment if managed correctly (Lantz et al., 2007; Sommer et al., 2003). In Vietnam, biogas systems not only provide gas for cooking but also improve the household environment by reducing problems with odor and flies (Huong et al., 2014). Traditionally, Vietnamese households have used solid animal manure to fertilize crops in fields and gardens. With many households now operating biogas digesters, they have shifted to the use of biogas effluent replacing raw or composted manure as a fertilizer of crops and vegetable (Chau, 1988; Francese et al., 2000); fish ponds and even as an animal food additive (Ngoc and Nhai, 2008; Tuyen et al., 2011). When building new houses, many Vietnamese pig farmers have installed new flush toilets that are connected to the biogas digesters (Huong et al., 2014). This is of concern since biogas effluent will most likely contain not only pathogens originating from pigs, but also a range of human pathogens that may be transmitted through contaminated water and foods when using biogas effluent as crop fertilizer or discharged indiscrinately into the external environment.

The objective of this study was therefore to evaluate the survival of *Salmonella* spp. and fecal bacterial indicators in biogas digesters operated by small-scale Vietnamese pig farmers and establish the serovar and antimicrobial susceptibility of the *Salmonella* spp. isolated.

Materials and methods

Selection and manure management practices of small-scale pig farms operating biogas digesters in Hanam province

The study was conducted and manure samples collected in the summer (June to August, 2011) and winter (December 2011 to February 2012), in Hoang Tan and Nhat Tan communes located in Kim Bang district, Ha Nam province in Vietnam. Lists of pig farmers with biogas digesters in the two communes were provided by the Department of Agriculture in Kim Bang district and the Center for Rural Water Supply and Environmental Sanitation of Hanam province. Farms were stratified by commune and whether their toilet was connected to the biogas unit or not. Eleven farms were selected from each stratum in Hoang Tay and 10 farms in Nhat Tan for questionnaire interviews. The questionnaire interview was done by first author of the person who managed manure (husband or wife) to investigate the current situation and hygiene aspect of manure management by farmers with biogas unit installed in Hoang Tay and Nhat Tan commune. The questionnaire interview collected information on the animal husbandry practices, management of the biogas digester, household toilet facilities as well as disposal and use of biogas effluent. In addition, farmers were asked whether health, fly and smell problems did change after installing biogas.

Collection of slurry and biogas effluent

Among the interviewed households that had an input tank connected to their biogas digester, six pig farms were randomly selected in each commune (three farms with toilet connected to the biogas digester and three farms without toilet connected to the digester) for microbiological analysis. Sampling was done during summer (June to August, 2011) and winter (December to February, 2012). In each season, households were visited six times. At each visit to the farm, one raw slurry sample was collected from the inlet tank placed before the biogas digester and one effluent sample was collected from the compensation tank of the biogas unit located after the biogas digester (Fig. 1). In total, 144 raw slurry and 144 effluent samples were collected. A sterilized 70-cm long spoon was used to mix the content of each tank thoroughly before sampling. About 1000 g of samples were collected from different parts of the tanks and poured into clean plastic buckets and approximately 200 g sample was transferred to a sterile glass bottle with screw cap. Samples were collected and kept in an insulated box with cooling elements and transported to the laboratory of the National Institute of Veterinary Research, Hanoi for bacteriological analysis which was initiated on the day of sampling. Samples were analyzed for *E. coli*, *Enterococcus* spp., spores of *Clostridium perfringens* and *Salmonella* spp.

Measurement of temperature, ammonia and pH

Manure samples from the biogas digester tank were collected during 12 sampling events for temperature, ammonium and pH analysis. Using handmade sampling equipment consisting of a plastic pipe with a diameter of 10 cm, a length of 2 m and connected to an inox stick with a length of 2.2 m, samples were collected inside the digester tank through the inlet pipe (Fig. 1). The sampler was cover by a lid to ensure that no manure from the input tank and input pipe would mix with the slurry in the digester tank. When the sampler reached the bottom of the digester tank, the lid was opened by pressuring with the inox stick. The lid remained open for one minute to allow enough slurry to be collected. The digestate in the sampler was poured into a clean sterile plastic bucket, mixed thoroughly and temperature measured directly by a normal hand-held thermometer. Approximately 500 g of digestate was transferred to a sterile 1000 mL glass bottle with screw lead. Samples were kept in an insulated box with cooling elements and transported to the laboratory of the National Institute of Soil and Fertilizer, Hanoi for pH and NH_4 analysis. pH was measured using a Hanna HI 8424 machine according to the Vietnamese standard method TCVN 6492-1999 (ISO 10523-1994) and NH_4 was mesuared according to TCVN 5988-1995 (ISO 5664-1984), and NH_3 concentrations were estimated as described by Christensen and Sommer (2013).

Enumeration of E. coli, Enterococcus spp. and Cl. perfringens spores

For the *E. coli* and enterococci analysis, a 10 g sub-sample (raw slurry and biogas effluent) was weighed in a sterile stomacher