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Impact of dairy manure pre-application treatment on manure composition, soil dynamics of antibiotic resistance genes, and abundance of antibiotic-resistance genes on vegetables at harvest

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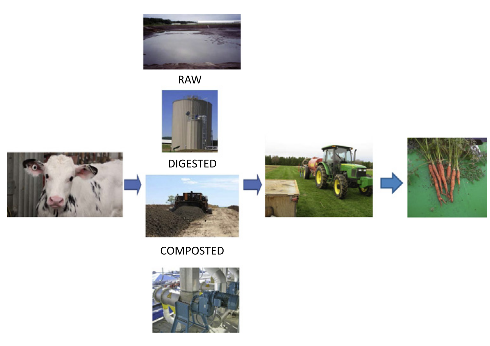
HIGHLIGHTS

- Effect of pre-treatment of manure on distribution of ARGs in soil and on vegetables was evaluated.
- No significant difference in persistence of ARGs following land application was observed.
- ARGs more frequently detected in soil receiving raw manure than in soil receiving composted manure.
- Vegetables grown in raw or digested manured soil are at risk of exposure to manure-born ARGs.

GRAPHICAL ABSTRACT



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ABSTRACT

Manuring ground used for crop production is an important agricultural practice. Should antibiotic-resistant enteric bacteria carried in the manure be transferred to crops that are consumed raw, their consumption by humans or animals will represent a route of exposure to antibiotic resistance genes. Treatment of manures prior to land application is a potential management option to reduce the abundance of antibiotic resistance genes entrained with manure application. In this study, dairy manure that was untreated, anaerobically digested, mechanically dewatered or composted was applied to field plots that were then cropped to lettuce, carrots and radishes. The impact of treatment on manure composition, persistence of antibiotic resistance gene targets in soil following application, and distribution of antibiotic resistance genes and bacteria on vegetables at harvest was determined. Composted manure had the lowest abundance of antibiotic resistance gene targets compared to the other manures. There was no significant difference in the persistence characteristics of antibiotic resistance genes following land application of the various manures. Compared to unmanured soil, antibiotic resistance genes were detected more frequently in soil receiving raw or digested manure, whereas they were not in soil receiving composted manure. The present study suggests that vegetables grown in ground receiving raw or digested

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manure are at risk of contamination with manure-borne antibiotic resistant bacteria, whereas vegetables grown in ground receiving composted manure are less so.

1. Introduction

The widespread development of resistance to antibiotics among human bacterial pathogens is leading to the loss of efficacy of clinically important medicines (Laxminarayan et al., 2013). The economic and human health consequences of this will be extremely serious if left unchecked (O'Neill, 2014). In response to the potential loss of treatment efficacy of all classes of clinically important antibiotics, many countries and international organizations concerned with human and animal health are developing action plans to mitigate antibiotic resistance (Commonwealth of Australia, 2015; ECDC, 2015; Government of Canada, 2015; U.S. White House, 2015). A key objective of these international initiatives is the mitigation of resistance development in the production of food animals, notably by reducing the use of antibiotics for growth promotion, prophylaxis or therapy (Durso and Cook, 2014; Pruden et al., 2013). Animal and poultry manures contain bacteria resistant to antibiotics, and the use of manures as a fertilizer in crop production represents a potential route of environmental exposure to antibiotic resistance genes (Agerso and Sandvang, 2005; Allen, 2014; Cook et al., 2014; Fahrenfeld et al., 2014; Heuer et al., 2011; Wang et al., 2015; Zhu et al., 2013). It is important in mixed agriculture that livestock and crop production systems be tightly coupled with respect to nutrient flow. Nutrients excreted by animals should be used as efficiently as possible to meet crop needs, and to avoid movement from the point of application to other environmental compartments leading to water or air quality problems. Several studies have evaluated the common practice of fertilizing crops with animal manures as a potential source of food and environmental contamination with antibiotic resistant bacteria (Cook et al., 2014; Heuer et al., 2011; Marti et al., 2013, 2014; Wang et al., 2015). In field experiments undertaken in London Ontario Canada, gene targets associated with resistance to selected antibiotics persisted in soils for several months following application of raw swine or dairy manure (Marti et al., 2013, 2014). Gene targets were more abundant on vegetable crops harvested from soil manured in the same growing season than from soil that was not manured. The study recommended that under our growing conditions, a period of time between manure application and crop harvest exceeding one growing season would reduce the potential exposure of produce to manure-borne antibiotic resistance genes. In season harvest does not allow sufficient time for dissipation of enteric bacteria and the antibiotic resistance genes that they carry (Marti et al., 2013). In addition to lengthy offset times, manure treatment options that reduce the burden of enteric bacteria prior to application is a management practice that should mitigate the transfer of antibiotic resistance genes from manured ground to crops.

In the present study, we sought to determine the impact of some manure treatment practices commonly employed in local commercial dairy farms on the composition of manure, the dynamics of selected gene targets following manure application, and the distribution of antibiotic resistance genes on vegetables at harvest. The microbial composition of animal manure is profoundly impacted by treatment, for example anaerobic or aerobic digestion, or composting (Côté et al., 2006; Topp et al., 2009). Pre-application treatment can reduce the abundance of viable antibiotic resistant bacteria, thereby presumably reducing the potential for persistence or exchange of antibiotic resistance genes following soil application (Chen et al., 2007). Overall, on-farm treatment of animal wastes prior to land application offers the potential to reduce the burden of antibiotic resistance genes enriched for in livestock in crop production systems (Pruden et al., 2013). In the present study, raw, anaerobically digested, mechanically dewatered, and dewatered composted dairy manure was applied to a series of field plots which were then sown to lettuce, carrots and radishes. The specific objectives were to: 1. evaluate the composition of the various manures with respect to the abundance of antibiotic resistance genes and bacterial composition; 2. compare the loading rate of antibiotic resistance genes to soil following the application of manure from commercial dairy farms that vary in how the waste is treated; 3. compare the rate of dissipation of selected gene targets in soil following application of the various manures; and 4. evaluate the distribution and abundance of antibiotic resistance genes carried on vegetable crops harvested from plots receiving the various manures.

2. Materials and methods

2.1. Field operations

Experiments were undertaken during the 2014 growing season on the Agriculture and Agri-Food Canada research farm in London, Ontario, Canada (42.984°N, 81.248°W). The air temperature and precipitation during the experimental period are presented in Fig. S1. Based on soil sample analysis and past cropping history, residual soil nitrogen and phosphorus was sufficient for crop needs, and therefore there was no application of inorganic fertilizers. Irrigation was not required for the 2014 season. Insect pressure was monitored during the growing season, and chemical or mechanical treatment was not necessary. Roundup herbicide (glyphosate) was applied at 4.5 L/ha for pre-plant weed burn down. Weed pressure during the growing season was subsequently monitored, and weeds were removed by hand hoeing, and at times mechanical cultivation. Manure applied in the spring of 2014 was obtained from two local dairy farms. One farm that had 150 Holstein cows supplied the raw manure slurry, and anaerobically digested manure digestate. The second farm had 70 Holstein and Jersey cows, and supplied the mechanically dewatered, and the mechanically dewatered then composted manures. The key characteristics of the manures are presented in Table S1. The raw and digested manure had high moisture contents and were applied as slurries, whereas the dewatered and composted manures had a much lower moisture contents and were applied as solids. Five plot areas of dimension 4 m × 105 m received no manure (*i.e.* untreated control), raw, composted, dewatered or anaerobically digested dairy manure. The plots were separated by 4 m borders. Application rates of solid manures were based on recommendations by the Ontario Ministry of Agriculture and Rural Affairs and the USDA. Plots were sub-divided into 4x6m plots for hand application of the dewatered and composted manure, using a scale and pails to apply material in each grid. The application rate for the dewatered manure was 5 t dry weight/ha, equivalent to 16.5 kg per 4 × 6m plot. The application rate for the composted manure was 5 t dry weight/ha equivalent to 14.5 kg per 4 × 6 m plot. All liquid application rates were based on manure content of crop-available nitrogen determined with an AgrosNquick test meter (Agros, Lidköping, Sweden). The raw and digested manures were applied using a Nuhn manure tanker (Sebringville, ON). The application rate for the liquid raw manure was 74,800 L/ha, and the application rate for the digestate was 80,410 L/ha. In all cases, immediately following application, manures were soil incorporated to a depth of 15 cm with two passes of a disk followed by three passes with a "S" tine cultivator prior to planting. Vegetable varieties planted were radish (*Raphanus sativus* variety Sora; 600 seeds per row spaced at 75 cm), carrots (*Daucus carota* variety Ibiza hybrid; 75-cm rows thinned at emergence), and lettuce (*Lactuca sativa* variety Summertime); 100 seeds