

Short Communication**UNDERSTANDING THE MANAGEMENT PRACTICES OF ANIMAL
MANURE AND ASSOCIATED RISKS OF TRANSFERENCE OF BACTERIAL
PATHOGENS TO CROP VEGETABLES****Neetoo H^{1*}, Goburdhun D¹, Ruggoo A², Pohoroo S¹, Pohoroo A¹ and K Reega¹****Huda Neetoo**

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ABSTRACT

Manure is commonly used in agricultural production in Mauritius, but little is documented on the local management practices. Animal manure, in particular, is a livestock waste that harbors enteric microorganisms which are potentially pathogenic to humans. The objectives of the study were therefore (i) to shed light on the management practices of manure among cattle and poultry farmers (manure producers) and carrot and lettuce growers (manure end-users) and any associated health risks and (ii) to determine the prevalence of human pathogens (diarrheagenic *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Clostridium perfringens*) in manure collected from farmers, vegetable crops fertilized with manure as well as manure-amended soil (MAS) used in crop cultivation. A survey was conducted through in-depth interviews with 16 producers and 36 end-users to gather data on their MMP and their perception of the health risks associated with manure handling. Samples of manure, MAS and vegetables were also microbiologically analyzed to enumerate and/or detect pathogens. Findings revealed that cattle and poultry manure was an important resource for many small-holder vegetable farmers in Mauritius. The manure distributors or end users had no negative perception of the use of untreated manure for vegetable cultivation and were generally unaware of any biosecurity risks arising from the improper handling or subsequent use of untreated manure. Microbiological analyses however showed that 100% of manure samples collected from cattle farms and 58% of the poultry litter samples tested positive for pathogenic *E. coli* with population ranging from 3.3 to 6.5 Log CFU/g. Manure-borne pathogens were generally undetectable in the analyzed vegetables hence indicating a low risk of foodborne infections. However, the systematic presence of pathogenic *E. coli* in cattle manure and frequent occurrence in poultry litter clearly point to a need for creating greater awareness amongst farmers on the occupational health risks associated with handling of raw or inadequately decomposed manure. This study therefore points to the health risks associated with enteric pathogens present in raw or untreated raw manure in Mauritius.

Key words: Manure Management, Pathogens, *E. coli*, *Salmonella*, Cattle, Poultry, Carrot, Lettuce, Mauritius



INTRODUCTION

Manure is any material that fertilizes land and includes feces, urine, and bedding from livestock (animal manure), residue or biomass from plants, as well as decomposed forms of either animal or plant residues (compost). Animal manure is being increasingly used in agriculture as it provides valuable nutrients for crops and helps improve crop yields [1]. While manure-amended soil (MAS) offers many advantages for cultivation, improper manure management practices (MMP) can have several drawbacks such as odor problems, water pollution, and several risks of diseases due to pathogens such as *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes* found in animal wastes [2 - 5]. Good animal husbandry practices, proper MMP, and health and safety awareness can alleviate or reduce the risks linked with manure handling, but these practices have yet to be documented for Mauritius. Therefore, the aim of this study was to understand the MMP of animal farmers and vegetable growers in Mauritius as well as shed light on the risks of pre-harvest contamination of vegetables by human pathogens originating from manure.

MATERIALS AND METHODS

A survey was conducted with 16 cattle and poultry breeders (manure producers) and 36 carrot and lettuce growers (manure end-users) and data collected by means of questionnaires. Manure samples (cattle and poultry) were also collected from seven animal farms while lettuce and carrot samples and MAS were collected from seven different produce farms around Mauritius. Nine samples of each type were collected from each farm and transported to the laboratory in an isothermal container. Samples of manure or MAS were pooled to form three composites of three sub-samples from which 25 g amounts were weighed. Carrot or lettuce samples were also pooled to form three composite samples and subsequently puréed using a sterilized blender. About 25 g of purée was mixed in 225 ml of 1% Buffered Peptone Water (Oxoid, UK) for 1 minute, using a stomacher (Seward, UK). Appropriate decimal dilutions were prepared in 0.1% sterile Buffered Peptone Water (HiMedia, India) and spread-plated on HiCrome *Listeria* Agar (HiMedia) for recovery of *L. monocytogenes* and plates incubated at 35°C for 24 h. Bluish green colonies were presumed to be *L. monocytogenes*. For *C. perfringens* enumeration, 1 ml of the homogenate was pour-plated with Iron Sulphite Agar (ISA; HiMedia) followed by an overlay of molten ISA. The plates were incubated anaerobically at 35°C for 48 h followed by enumeration of any black colonies formed. *Salmonella* was enumerated by plating appropriate dilutions on Xylose Lysine Tergitol-4 (XLT-4; HiMedia) and plates incubated at 35°C for 24 h. In addition, samples were also enriched by incubating the stomachate at 35°C for 24 h followed by secondary enrichment in Rappaport-Vassiliadis broth at 42°C for 18 h. The broth samples were then streaked on XLT-4. Dark-centered pink colonies were presumed to be *Salmonella* and any colonies formed were confirmed using biochemical tests and commercial lateral flow immunoassays (Reveal 2.0, Neogen). For the detection of pathogenic *E. coli* (PEC), 25 g samples of manure were homogenized with 225 ml of mTSB [6], followed by spread-plating on HiCrome EC O157:H7 (HiMedia). The homogenate was enriched and subsequently streaked onto HiCrome EC O157:H7 agar. Plates were incubated at 35°C for 24 h. Light pink to purple colonies were interpreted as PEC. Survey data collected was analyzed using Microsoft Excel 2013 and reported as percentages of manure



producers or end-users questioned during this survey. Microbial population data were analyzed, and log-transformed using Microsoft Excel 2013.

RESULTS AND DISCUSSION

Most of the cattle farms visited (88%) stored manure in an uncovered stockpile on the ground, near or outside the barns. There was no set-up for effluent discharge in any of the surveyed barns. Litter of broilers and layers generated by poultry farms typically comprised of a dry mixture of chicken droppings and bedding. All poultry farmers indicated that they stored deep-litter poultry manure on concrete surfaces in the animal house itself. Most farmers did not store manure for any defined period and used them as and when needed thus underscoring the risks when using or handling raw or inadequately decomposed manure. While 60% of the manure producers claimed to treat the manure before selling, the rest did not apply any treatment. Moreover, none of the livestock breeders indicated having received any training on the management of manure. The most popular form of “treatment” involved mixing manure with straws, leaving the manure mix in open-air or waiting for it to dry up and decompose. As soon as the manure was deemed sufficiently dry, it was picked up by a “middleman”, also referred to as the “collector” and sold to vegetable growers. 56% of growers reported applying solid animal manure to crops in its pure form while 22% and 15% of farmers used manure mixed with chemical fertilizers and water, respectively. Poultry and cattle manure were more commonly used by carrot and lettuce growers, respectively. There were however no standard operating procedures (SOPs) adopted by farmers for application of manure.

In fact, most farmers (89%) thought that animal wastes were safe and free of disease-causing microorganisms. Moreover, most farmers (93%) thought that manure carried little to no occupational health risks while a minority acknowledged the risks of zoonotic disease transmission. Microbiological analyses however showed that PEC was detected in 100% and 58% of cattle manure and poultry litter samples respectively with a mean population of 6.5 and 3.3 Log CFU/g but absent in vegetables. *Salmonella* sp. was generally undetectable in all manure, MAS, and vegetables samples except for one sample of poultry litter, MAS, and lettuce. *L. monocytogenes* was frequently isolated from manure (2.3 – 3.9 Log CFU/g) and MAS (2.9 – 3.3 Log CFU/g) but undetected in vegetables (Table 1). *Clostridium* spp. on the other hand was systematically undetectable in all sample types.

CONCLUSION

Although manure-borne pathogens were generally absent in the produce tested, there is nevertheless a need for sensitization of farmers on the health risks associated with pathogens present in raw or inadequately treated manure.

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Conflict of Interest

The authors declare that they have no conflict of interest.



Table 1: Microbiological Parameters of Manure, Vegetables, and Manure-amended Soil

Parameters	Cattle Manure	Poultry Manure	Lettuce	Lettuce MAS	Carrot	Carrot MAS
Pathogenic <i>E. coli</i> Density (Log CFU/g)	6.5 ± 0.30	3.3 ± 1.78	< 2 (2/21)	< 2 (3/21)	< 2 (0/21)	< 2 (1/21)
<i>Salmonella</i> Density (Log CFU/g)	< 2 (0/21)	2.3 ± 0.91	2.0	< 2 (0/21)	< 2 (0/21)	2.6 ± 1.74
<i>Listeria</i> spp. (Log CFU/g)	3.9 ± 1.44	2.3 ± 0.84	< 2 (0/21)	3.3 ± 1.26	< 2 (0/21)	2.8 ± 1.15
<i>C. perfringens</i> (Log CFU/g)	< 1 (0/21)	< 1 (0/21)	< 1 (0/21)	< 1 (0/21)	< 1 (0/21)	< 1 (0/21)

Abbreviation: MAS - Manure-Amended Soil

Values represent the means of 21 data points

< 1 or < 2 Log CFU/g represent the Limit of Detection by the plating methodology

Numbers in brackets represent the number of samples testing positive after enrichment out of 21



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