Effects of metal and metalloid pollutants on the microbiota composition of feces obtained from twelve commercial pig farms across China

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HIGHLIGHTS

- 50% of global pig production is in China.
- Waste is often used for fertilizer.
- Al, Mn, Cu, Zn, and Fe pollution were frequently detected in pig feces.
- Feces from pigs in the fattening stage contained more bacteria and disease genes.

GRAPHICAL ABSTRACT
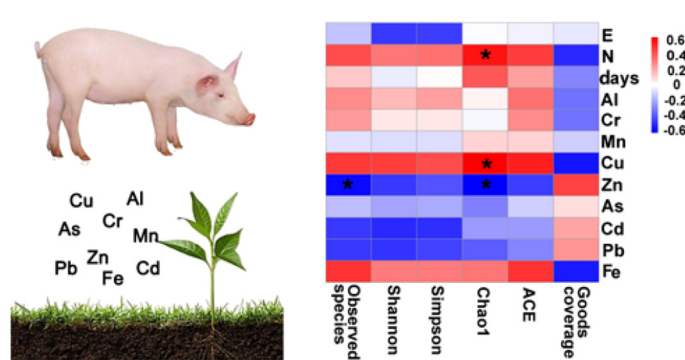
ABSTRACT

Understanding the metal and metalloid contamination and microbiota composition of pig feces is an important step required to support the design and implementation of effective pollution control and prevention strategies. A survey was implemented in 12 locations across China to investigate the content of metals and metalloids, and the main composition of the microbial communities of commercially reared pigs during two growth periods, defined as the early (Q group) and the later fattening growth phases (H group). These data showed widespread Al, Mn, Cu, Zn, and Fe pollution in pig feces. The concentration of Zn in the Q group feces was nearly two times higher than the levels measured in the H group. The microbial composition of the Q group exhibited greater richness of operational taxonomic units (OTUs) and fewer bacteria associated with zoonotic diseases compared with the microbial composition of the H group. Spearman rank correlation analysis showed that Cu and northern latitudes had a significant positive effect on the richness of bacterial communities in pig feces. Zn and Cd exhibited the biggest impact on microbial community composition based on canonical correspondence analysis. Functional metagenomic prediction indicated that about 0.8% genes present in the pig feces bacteria community are related to human diseases, and significantly more predicted pathogenic genes were detected in the H group than in the Q group. These results support the need to monitor heavy metal contamination and to control for zoonotic pathogens disseminated from pig feces in Chinese pig farms.

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1. Introduction

With the economic boom and rising incomes, China's pig industry has increased significantly over the past decades, making up nearly 50% of global pig production (Bai et al., 2014). However, the development of large-scale and highly concentrated pig farming results in the production of large volumes of feces (an estimated 618 billion kg of feces is produced each year in China) presenting multiple challenges to environmental and public health (Larson, 2015; Zhang et al., 2014). Pollutants found in pig feces include pathogens, metals and metalloids (metal(loid)s), and veterinary antimicrobial agents (Trawińska et al., 2015). The pollutants found in pig feces may vary for different growth stages due to the high dependence on variations in the feed composition (Sun and Wu, 2013).

Metal(loid)s are added to pig feed formulations for growth promotion and disease prevention. Most of these elements (some up to 95%) are subsequently excreted in the feces and are a key source of heavy metal pollution applied to agriculture land (Ding et al., 2017; Yin et al., 2017). The metal(loid)s that accumulate in farm plants through soil-food chain transfer may affect the health of animals and humans (Lu et al., 2015). Approximately 40% of the total annual inputs of Zn and Cu and 11% of Cd inputs to agricultural land derive from animal feces (Wu et al., 2013). This is especially a concern in places like Zhejiang Province, China, where many of the vegetables that are grown locally are contaminated by metal(loid)s, which may adversely affect public health (Wu et al., 2013).

Contamination of animal feces with microbes, especially pathogenic bacteria, may also threaten human and animal health (Andres and Davies, 2015; Sun and Wu, 2013). The most frequently isolated pathogenic bacteria from pig feces include diarrheogenic *Escherichia coli*, *Staphylococcus aureus*, *Shigella* spp., *Yersinia enterocolitica*, and *Salmonella* (Liu et al., 2016; Andres and Davies, 2015). Insufficient efforts to limit microbial risks are likely to correlate with a higher prevalence of pathogen infection, increased cross-contamination of carcasses processed at slaughterhouses, and increased human zoonosis (Andres and Davies, 2015). Therefore, it is important to assess the prevalence and composition of microbial communities in pig feces in China to evaluate their potential pathogenic risk.

Several factors can influence pig intestinal bacterial communities, including the growth stage of the animal, the use of feed additives (including antibiotics and metal(loid)s), health status, raising process management, and geographical factors (Liu et al., 2015; Zhao et al., 2015). However, little is known about how these factors that can influence the composition of these communities during different stages of pig rearing. In particular, how the presence of metal(loid)s may influence fecal bacterial communities during these different stages is poorly understood, although it is known that metal(loid)s can have significant effects on bacterial growth in natural environments (Heinlaan et al., 2008; Harrington et al., 1998). Overall, it is important to gain a better understanding of the factors that can influence fecal bacterial communities and better insight into the effects on the bacterial communities of metal(loid) content, stages of pig growth, and geographical factors throughout the pig-raising process.

With the dramatic expansion of pig production occurring only recently, there are still only few laws and regulations designed to control pig feces pollution in China. Additionally, there is limited data revealing the impact of risk factors to human health, which limits the ability to craft effective legislation. Understanding the relationship between metal(loid)s contamination and the microbial composition in pig feces is an important step toward the development of effective pollution control measures and better strategies to protect public health in China. Earlier studies lacked the depth to provide a comprehensive interpretation or description at the micro-level (pathogen pollution and toxic components) or the macro-level (pollutant loads, pollution control, and monitoring mechanisms) for pollution from pig farming (Sun and Wu, 2013). Thus, a more detailed pollution survey of pig production sites is required to assist in the formulation of appropriate policy.

The specific objective of this study was first to investigate the extent of metal(loid) contamination and assess microbial community diversity through high-resolution sequencing. This was performed for full-length 16S rRNA sequences using the Pacific Biosciences (PacBio) platform for samples isolated from pig manure from 12 farms scale across China. Analysis of the sequencing information was then related to the growth phase of pigs, concentrations of metal(loid)s, and the location of the farms where the samples were obtained. These data allowed the comparison of the differences in microbial communities and pathogenicity risks between early and later phases of pig growth.

2. Materials and methods

2.1. Sampling location and collection

The fecal sample collection procedure was approved by the Animal Ethics Committee of the China National Food Safety Risk Assessment Center.

Pig fecal samples were collected in November 2015 and January 2016 from 12 surveyed farms in 12 provinces or municipalities in China and mixed into 14 total samples. Each mixed sample included 20 collected samples from each location, described in more detail below. The sampling sites, farming operation scales, global position system (GPS) location, and pig information are shown in Fig. 1, Tables S1, and S2. According to the number of sows raised, pig farms were classified as large (above 800 sows), medium (200–800 sows), and small (below 200 sows) scales. At each sampling farm, four separate pig houses were selected randomly for sampling, and five fresh feces samples were taken from each house and then pooled for DNA isolation. These twenty samples obtained from each farm were then combined into a single representative sample for each farm and subjected to analysis.

For the 14 feces samples, nine were collected during the early phases of pig growth (animals were between 50 and 90 growing days in age, and this cohort was denoted as the Q group), and five samples were collected during the later fattening growth phase (between 105 and 130 growing days, denoted as the H group). To directly compare the effect of pig growth phase for the same location, two pairs of samples, HEBQ/HEBH (taken from early/later phases of pig growth phase in a farm of Hebei Province) and SHQ/SHH (taken in Shanghai City), were collected and used to directly assess effects of pig life stage. The temperatures of all the pig houses were 15–23 °C.

Samples from all locations were transported back to the laboratory within 3 h at 4 °C in a container. Upon returning to the laboratory, the feces samples were immediately transferred to −20 °C prior to metallic and metalloid elemental determination and DNA extraction.

2.2. Metal(loid) content determination

The amounts of metals isolated from the soluble fraction of the pig feces samples were determined by inductively coupled plasma mass spectrometry (ICP-MS). The amounts of aluminium (Al), cadmium (Cd), chromium (Cr), copper (Cu), ferrum (Fe), manganese (Mn), lead (Pb), zinc (Zn), and metalloid arsenic (As) were analyzed in their more relevant soluble forms because the dissolved fractions have higher transport capacity and are more easily assimilated and bioavailable to vegetation and animals (Soriano et al., 2012).

This methodology for the metal(loid) content quantitative analysis was described previously (Finger et al., 2017). Briefly, the total concentrations of metals and metalloid were determined by ICP-MS using an Agilent 7700× (Agilent Technologies, USA) after microwave digestion of 0.5 g of representative feces sample. The date was expressed as the mean ± SD of replicate values where indicated.