

Johanna Santamaría · Gary A. Toranzos

Enteric pathogens and soil: a short review

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Abstract It is known that soil is a recipient of solid wastes able to contain enteric pathogens in high concentrations. Although the role of soil as a reservoir of certain bacterial pathogens is not in question, recent findings show that soil may have a larger role in the transmission of enteric diseases than previously thought. Many of the diseases caused by agents from soil have been well characterized, although enteric diseases and their link to soil have not been so well studied. Gastrointestinal infections are the most common diseases caused by enteric bacteria. Some examples are salmonellosis (*Salmonella* sp.), cholera (*Vibrio cholerae*), dysentery (*Shigella* sp.) and other infections caused by *Campylobacter jejuni*, *Yersinia* sp. and *Escherichia coli* O157:H7 and many other strains. Viruses are the most hazardous and have some of the lowest infectious doses of any of the enteric pathogens. Hepatitis A, hepatitis E, enteric adenoviruses, poliovirus types 1 and 2, multiple strains of echoviruses and coxsackievirus are enteric viruses associated with human wastewater. Among the most commonly detected protozoa in sewage are *Entamoeba histolytica*, *Giardia intestinalis* and *Cryptosporidium parvum*. This article reviews the existing literature of more than two decades on waste disposal practices that favor the entry of enteric pathogens to soil and the possible consequent role of the soil as a vector and reservoir of enteric pathogens.

Keywords Soil-borne diseases · Enteric pathogens · Enteric disease · Enteric disease outbreaks · Solid waste disposal

Introduction

Humans are in contact with soil permanently, either directly or indirectly via food, water and air; and thus soil may act as a vector and source of important human disease agents. Although many of the diseases associated with soils have been well characterized and studied, enteric diseases and their link to soil have been understudied and possibly underestimated. In order to clarify this connection, diseases associated with soil have been classified depending on the origin [39] of the etiological agent as follows: (1) soil-associated diseases which are caused by opportunistic or emerging pathogens that belong to the normal soil microbiota (e.g. *Aspergillus fumigatus* is a very common fungus occurring in soils and can infect the lungs via inhalation of spores), (2) soil-related diseases, which result in intoxication from the ingestion of food contaminated with entero- or neurotoxins (*Clostridium botulinum*, *C. perfringens* and *Bacillus cereus* are some examples of these pathogens), (3) soil-based diseases caused by pathogens indigenous to soil (which include *C. tetani*, *B. anthracis*, and *C. perfringens*) and (4) soil-borne diseases caused by enteric pathogens which get into soil by means of human or animal excreta. Enteric pathogens transmitted by the fecal–oral route are bacteria, viruses, protozoa and helminths.

Gastrointestinal infections are the most common diseases caused by enteric bacteria. Some examples are salmonellosis (*Salmonella* sp.), cholera (*Vibrio cholerae*), dysentery (*Shigella* sp.) and other infections caused by *Campylobacter jejuni*, *Yersinia* sp. and *Escherichia coli* O157:H7 and many other strains. *E. coli* O157:H7 successfully causes infections because of its low infectious dose (ID), which can be as few as ten cells [28].

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J. Santamaría · G.A. Toranzos (✉)
Environmental Microbiology Laboratory,
Department of Biology, University of Puerto Rico,
P.O. Box 23360, 00931 San Juan, Puerto Rico
E-mail: gtoranzo@nsf.gov
Tel.: +1-787-7515708
Fax: +1-787-7643875

The most commonly detected protozoa in sewage include *Entamoeba histolytica*, *Giardia intestinalis* and *Cryptosporidium parvum*. These pathogens cause diarrhea and the illness can result from the ingestion of just ten cysts/oocysts or less [11, 13]. *Giardia* is the parasite most commonly detected in gastroenteritis patients in the United States [15]. Some of the most commonly detected helminths in wastewater are *Ascaris lumbricoides*, *Necatur americanus*, *Trichuris trichiura* and *Strongyloides stercoralis* [38] and, although they are not commonly detected in patients in industrialized countries, they remain as major etiological agents of disease in countries with poor sanitary facilities.

Sources of soil contamination by enteric pathogens

As a result of the intrinsic characteristics of soil, any member of the allocthonous or indigenous microbiota will eventually end up in an aquatic environment or be dispersed in aerosols. There is a concern about a possible increase in soil-borne diseases in human populations, given the successful land disposal practices of sewage and sewage sludges that result from wastewater treatment. These practices may favor the entry of considerable concentrations of enteric pathogens into soil, because large amounts of these solids are applied to lands or disposed of in landfills. More than three million gallons of sewage effluent from more than 3,000 land-treatment sites and 15 million septic tanks were applied to land every day 20 years ago [16]. Currently, it is estimated that more than seven million dry tonnes of sewage sludge are produced annually and 54% of this is applied to soil [7].

A variety of treatment methods, such as composting, aerobic and anaerobic digestion, alkaline stabilization, conditioning, dewatering and heat drying, are used in wastewater-treatment plants to reduce pollutants and to destroy pathogens. Sludge is the first product of this treatment and, if additional treatment is given in order to reduce the pathogen concentrations to specific levels as indicated below, the material becomes a "biosolid". Biosolids are classified as either class A or class B, in categories established by the Environmental Protection Agency (EPA), based on the following microbiology criteria: Class A biosolids must have a concentration of thermotolerant coliforms below 1,000 colony-forming units (CFU)/g dry weight (dw) by the most probable number (MPN) method, a *Salmonella* concentration of less than 4 CFU/g dw, an enteric virus concentration of less than four plaque-forming units/g dw and less than four viable helminth eggs/g dw. Class A biosolids can be applied to lawns and home gardens and given away to the public in bags or other containers. In general, they are used like any commercial fertilizer.

Class B biosolids are required to have a geometric mean concentration of thermotolerant coliforms of less than 10^6 CFU/g dw. Class B biosolids may contain *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter*,

Cryptosporidium, *Giardia*, Norwalk virus and enteroviruses [21]. Its use is restricted to land application, forest lands, reclamation sites and, for a period of time, access is limited to the public and to livestock grazing and the harvest schedule is controlled. This time period allows for the natural die-off of pathogens in the biosolids.

Although the "Standards for use and application of sewage sludge" (40 CFR, part 503, promulgated by the EPA) was created for the safe use of these biosolids, these regulations were based on scientific information for which no risk assessment studies had been carried out. There is concern about the effect that the disposal of these solids may have on public health because: (1) the fate of these enteric microorganisms in the soil is not well understood and thus they may be a contamination source for food or surface- and groundwater, (2) the infectious dose of some pathogens is low (such as in the case of *Giardia*, *Cryptosporidium*, enteric viruses) and this could imply a high risk, especially in special populations, such as the immunocompromised and the elderly, (3) there is a possibility of regrowth of pathogenic bacteria [14, 41], (4) the presence of indicator bacteria, such as coliforms, which is used as an index of safety, does not accurately predict the presence of pathogens and (5) many diseases may be due to unknown agents and the methods for their detection have not yet been developed [20]. In fact, one of the major problems in assessing the microbiological safety of biosolids is precisely the lack of robust and sensitive methods for the detection of pathogens.

In developing countries, untreated domestic wastewater is an important source of enteric pathogens to soil because it is used in agricultural irrigation. This presents a high risk to farm workers and to consumers of food products irrigated with wastewater [34].

Other practices that favor the entry of considerable amounts of enteric pathogens into the soil environment are the use of human and animal excreta as manure and the inadequate disposal of human excreta in national parks and in general in areas where toilets are not provided [5]. Feachem et al. [8] present information on the survival times of some excreted pathogens in soil and on crop surfaces. Enteroviruses, thermotolerant coliforms and *Salmonella* spp persist less than 20 days, *V. cholerae* persists less than ten days and helminth eggs may persist for several months. Blum and Feachem [3] reviewed the existing epidemiological evidence of the agricultural use of excreta and they concluded that crop fertilization with untreated excreta causes significant infections with intestinal nematodes and bacteria in consumers and field workers. Excreta treated by freezing or high temperature seem to have lower concentrations of pathogens, although many of them can survive these treatments, most likely as a result of the high concentration of organic material around them serving as an insulator. For example, *Salmonella* cells are not inactivated by freezing and are relatively resistant to drying [14, 26], while helminth egg concentrations are reduced by these treatments but are not completely eliminated. Taking

into account that the cyst infectious dose is low, composting practices do not completely eliminate the risk of infection.

Municipal solid waste (MSW) may be another source of enteric pathogens to soil because most landfill sites were constructed without a leachate collection system. This leachate may contain viruses and bacteria [10] which can percolate through soil and contaminate groundwater. In 1993, 62.4% of 205×10⁶ t of MSW generated in the United States were sequestered in landfills [23] and 50% of these sites were in the vicinity of water wells [2]. Enteric pathogens in MSW come from the excreta present in disposable diapers, pet feces, food waste and sewage sludge [10].

On-site soil disposal systems (OSDSs) treat domestic water for 20% of the United States population and could also result in soil, and consequently groundwater, contamination [29].

Fate of enteric pathogens in soil

Soil moisture favors the survival of viruses and bacteria. Reductions in bacterial and viral population densities are observed under dry soil conditions. Clays favor the adsorption of microorganisms to soil particles and this further reduces the die-off rates [12, 40]. Clays protect bacterial cells, and possibly viral particles, by creating a barrier against microbial predators and parasites [27]. Thus, the rates of enteric pathogen survival are lower in sandy soils with a low water-holding capacity. pH affects the adsorption characteristics of cells, so inactivation rates in acidic soils are lower. Increases in cation concentrations also result in increased adsorption rates, consequently affecting microbial survival. Soluble organics increase survival and, in the case of bacteria, may favor their regrowth when degradable organic matter is present. Lower temperatures favor bacterial and viral survival. In laboratory studies, as the temperature increased from 15 °C to 40 °C, the inactivation rate increased significantly for poliovirus type 1 [32]. The ultraviolet light from the sun inactivates viruses on the surface of the soil but viruses in deeper layers are not affected [12].

Microbial movement in soils is dependent on the water saturation state. Microorganisms move rapidly under saturated conditions, but only for a few centimeters, because microorganisms are in close contact with soil particles, promoting the adsorption of microorganisms onto the soil particles. When soil is saturated, all pores are filled with water, allowing microorganisms to pass through the soil. Thus, soil texture controls, in part, the movement of microorganisms, because fine-grained soils avoid movement while coarse-grained soils promote it [1, 31]. Another important environmental factor affecting microbial movement is rainfall. It can result in pathogen spread by runoff from places where manure or biosolids have been applied or by leaching through the soil profile. It is known that bacterial and

viral groundwater contamination increases during heavy rainfall. The presence of coliforms was monitored for 9.4 m and 153.3 m wells. Coliforms were detected in both shallow and deep wells, with bacterial contamination coinciding with the heaviest rainfalls [12]. In Quebec, Canada, human and pig enteroviruses were isolated from 70% of the samples collected from a river. The contamination source was attributed to a massive pig-raising activity in the area [25].

Among enteric pathogens, viruses seem to be some of the most resistant to inactivation. To study the virus transport from septic tanks to ground and surface-water resources, several experiments were carried out using vaccine poliovirus type 1 as the seed [9]. Seeded viruses were detected up to 50 m from the septic tank, indicating they could easily travel through silt loam; and they were also detected in a nearby lake at 43 days and 71 days after seeding. In another field study in a farm that had received anaerobically digested sludge for 7 years, it was possible to detect viral nucleic acid sequences at points vertically and laterally displaced from sludge injections [33]. However, it should be noted that the survival and transport of viruses in soil is highly dependent on the type of virus [9].

Outbreaks of soil-borne diseases

Annual summaries of food-borne and water-borne disease outbreaks published by the Centers for Disease Control show that, in the past decade, there was an increase in food-borne and water-borne outbreaks caused by enteric pathogens. It is possible that the water and food contaminations were related to the practices mentioned above. For example, in the United States, water-borne diseases caused by contaminated ground water increased in the past decade [6, 20]. Several outbreaks associated with bacteria, viruses and protozoa were attributed to OSDSs. A cryptosporidiosis outbreak in the United Kingdom with 47 reported cases had a strong statistical correlation with two groundwater sources. One of them had a cross-connection with an OSDS and the other received surface runoff from a nearby grazing pasture during heavy rainfalls [4]. A shigellosis outbreak affected 1,200 people in Florida; and the contamination was traced to a church's OSDS [39]. A Norwalk virus outbreak in Washington affected 72% of the students and teachers at a grade school [36]. In an outbreak in Wyoming, 157 persons were infected with *E. coli* O157:H7 and the source was assumed to be fecal contamination by wildlife near a spring well [20].

Fruits and vegetables frequently come in contact with soil post-harvest and thus may become contaminated with soil enteric pathogens present in sewage sludge or manure spread. There are many examples of food-borne outbreaks traced to fresh fruits and vegetables. One of the first cases of infection with *E. coli* O157:H7 linked to the use of animal excreta as manure was with an ovo-vegetarian woman who consumed almost exclusively the

food produced in her garden, in which she used the manure from her own cow as a fertilizer [22]. In 1970, an outbreak occurred as a result of the ingestion of vegetables irrigated with wastewater. Further studies indicated that *V. cholerae* was present in the irrigated soils [30].

Unpasteurized juice and cider contaminated with *E. coli* O157:H7, *Salmonella* [20] and *Cryptosporidium* [19] have been implicated in several outbreaks in the United States and Canada. Fruit juice and cider may become contaminated as a result of the fruit falling to the ground and coming in contact with soil which may contain pathogens from animal excreta or sewage sludge used as fertilizer. Unpasteurized juice has been associated with at least 15 food-borne illness outbreaks since 1900 [24]. Several reports of raw sprouts have also been linked to outbreaks of food-borne illness with *E. coli* O157:H7 [18] and *Salmonella* [35] as the etiological agents.

Ingestion of soil, or geophagia, is another way in which humans, and especially infants and young children, can get infected with enteric pathogens [37]. Although geophagia is the voluntary ingestion of soil, involuntary ingestion as a result of wind could present a risk to immunocompromised individuals and other special populations [17, 37].

Concluding remarks

Data are scarce and in fact are mostly non-existent as far as the role of soil as a vector or reservoir of enteric infections for humans and animals is concerned. In the absence of data, it would be impossible to carry out risk assessment studies to determine the true danger of the presence of enteric microorganisms in soil. In any case, it seems obvious to assume that any microorganisms present in soil, either allocthonous or autocthonous, will eventually end up in the water or air as a result of run-off and wind. Thus, the role of soil when carrying out studies on enteric diseases cannot be obviated.

Studies are needed to determine the true risk of enteric infections related to soil ingestion. Among the studies that need to be carried out are: (1) the survival of enteric microorganisms in different types of soil, (2) the ability of different types of soils to either protect or inactivate pathogenic microorganisms, (3) the ability of pathogens to invade and colonize vegetables that are eaten raw, (4) the development of methods for the detection and quantitation of enteric pathogens in soils and (5) risk assessment.

During enteric disease outbreaks, most studies focus on the role of either water or food as the source of the pathogens. However, the intermittent presence of enteric pathogens in, for instance, water makes their detection difficult. However, if soil is in fact an important source of microorganisms, it may be easier to detect them in this matrix when methods are developed and standard-

ized. The development of robust detection methods and studies on the ecology of enteric pathogens in soils should be a priority; and without these data, a true characterization of public health risk as a result of direct or indirect exposure to soils will be impossible.

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