Contamination with bacterial zoonotic pathogen genes in U.S. streams influenced by varying types of animal agriculture

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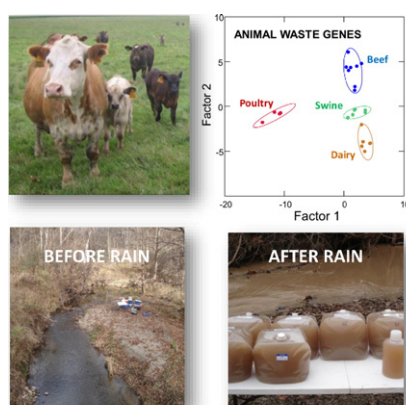[c] U.S. Geological Survey, 12201 Sunrise Valley Drive, Reston, VA 20192, United States
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HIGHLIGHTS

- We studied 21 bacterial pathogen genes in 19 small animal-agriculture watersheds.
- Animal-waste gene profiles were animal-specific regardless of geographic location.
- Stream water gene profiles could be discriminated by the watershed animal type.
- Gene profiles may help resolve land-use influence on microbiological water quality.
- Routine pathogen gene studies could address transport, fate and potential health risks.

GRAPHICAL ABSTRACT

ABSTRACT

Animal waste, stream water, and streambed sediment from 19 small (<32 km^2) watersheds in 12 U.S. states having either no major animal agriculture (control, $n = 4$), or predominantly beef ($n = 4$), dairy ($n = 3$), swine ($n = 5$), or poultry ($n = 3$) were tested for: 1) cholesterol, coprostanol, estrone, and fecal indicator bacteria (FIB) concentrations, and 2) shiga-toxin producing and enterotoxigenic *Escherichia coli*, *Salmonella*, *Campylobacter*, and pathogenic and vancomycin-resistant enterococci by polymerase chain reaction (PCR) on enrichments, and/or direct quantitative PCR. Pathogen genes were most frequently detected in dairy wastes, followed by beef, swine and poultry wastes in that order; there was only one detection of an animal-source-specific pathogen gene (*stx1*) in any water or sediment sample in any control watershed. Post-rainfall pathogen gene numbers in stream water were significantly correlated with FIB, cholesterol and coprostanol concentrations, and were most highly correlated in dairy watershed samples collected from 3 different states. Although collected across multiple states and ecoregions, animal-waste gene profiles were distinctive via discriminant analysis. Stream water gene profiles could also be discriminated by the watershed animal type. Although pathogen genes were not abundant in stream water or streambed samples, PCR on enrichments indicated that many genes were

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from viable organisms, including several (shiga-toxin producing or enterotoxigenic *E. coli*, *Salmonella*, vancomycin-resistant enterococci) that could potentially affect either human or animal health. Pathogen gene numbers and types in stream water samples were influenced most by animal type, by local factors such as whether animals had stream access, and by the amount of local rainfall, and not by studied watershed soil or physical characteristics. Our results indicated that stream water in small agricultural U.S. watersheds was susceptible to pathogen gene inputs under typical agricultural practices and environmental conditions. Pathogen gene profiles may offer the potential to address both source of, and risks associated with, fecal pollution.

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1. Introduction

In the United States, the Total Maximum Daily Load (U. S. Environmental Protection Agency [USEPA], 2001) watershed management process defines water quality impairments. Pathogens are the number one reported impairment; however, pathogen impairment is assessed not by evaluating actual pathogens but by determining whether the concentration of fecal indicator bacteria (FIB) is acceptable with regard to applicable water standards (such as for recreation, shellfish harvesting, or drinking water; USEPA, 2001). FIB typically include fecal coliform, *Escherichia coli* (*E. coli*) and/or enterococci bacteria. For water quality purposes, enterococci are defined by growth on the recommended medium (USEPA, 2006). Although FIB provide a simple and inexpensive test, FIB do not indicate the source of pollution, have no specific relationship to pathogens, and unless epidemiological studies are performed, do not indicate health risk (Field and Samadpour, 2007; Harwood et al., 2014). In recent years, advances in molecular methods now allow for rapid analysis in environmental samples of pathogen-specific genes, genes for bacteria uniquely associated with specific animal sources (microbial source tracking), and for analysis of the molecular signature of entire microbial communities (see reviews in Harwood et al., 2014; Samhan et al., 2015; Tan et al., 2015). Routine testing for the types of genes that allow microorganisms to cause disease in humans or animals (virulence genes) could provide improved information on the occurrence, fate, transport, survival, and environmental sources of pathogens. Although further testing for viable organisms capable of actually causing disease would be required in order to establish risk to human or animal health, knowledge of the potential for occurrence and distribution of pathogens would be useful in many areas of environmental management.

Pathogens of concern to human health occur in fecal wastes from humans, companion animals, wildlife, and livestock (Atwill et al., 2012; USEPA, 2009a, 2009b). Pathogen contamination of water may result from land application of animal manure, as well as from water access by animals and runoff of animal droppings during rain events (Atwill et al., 2012; USEPA, 2013). Although there are numerous zoonotic (transferred between humans and animals) bacterial and protozoan pathogens of human health concern, livestock-associated *E. coli* O157:H7, *Campylobacter* and *Salmonella* are considered priority pathogens along with the protozoa *Cryptosporidium parvum* and *Giardia duodenalis* based on their ability to be waterborne and cause significant illness in humans (Atwill et al., 2012). Watershed-wide studies of the distribution of priority zoonotic bacterial pathogens, using growth-based methods, or gene-based methods, have been conducted (e.g., Drozd et al., 2013; Ferguson et al., 2013; Fremaux et al., 2009; Jiang et al., 2007; Jokinen et al., 2011; Vereen et al., 2013; Walters et al., 2011). Characterizing the contributions of bacterial pathogens from animal agricultural practices and sources in large-scale watershed settings is confounded by the presence of multiple animal types and human influences (e.g., wastewater treatment plant effluents, septic systems, street runoff; McAllister and Topp, 2014). Studies conducted in well-controlled laboratory or small-scale field experiments (Bradford et al., 2013) have elucidated the complex array of soil, weather, and agricultural management variables that may influence pathogen fate and transport in agricultural environments, but are limited in

transferability to the watershed scale. Thus, information is still needed on the degree to which common livestock agricultural practices, animal types, and environmental variables influence pathogen occurrence in streams under typical watershed conditions.

In this study we evaluated concentrations of the FIB *E. coli* and enterococci, fecal indicator chemicals (cholesterol, coprostanol, and estrone), and the presence of genes indicating priority zoonotic bacterial pathogens, as well as *Shigella*, a uniquely human-source pathogen, and the STh gene indicating human sources, in stream water and sediment in 19 small (<32 km^2) watersheds in 12 U.S. states having either no animal agriculture (control), or a single dominant animal (beef, dairy, swine, or poultry). For method comparison, we tested genes both with and without prior growth-based enrichment of the pathogens. Animal manure from the watershed was also tested and stream water samples were collected before and after rainfall events. The study was part of a larger survey of inputs to streams from animal agriculture, that also investigated stream water estrogenicity and effects on fish (Alvarez et al., 2013; Cavallin et al., 2014). The study design was uncomplicated by mixed land uses or anthropogenic fecal waste sources, occurred at an intermediate scale between the field and large watershed scales, reflected typical agricultural practices across the U.S., and included replication of animal-type watersheds and comparison with control watersheds.

2. Methods and materials

2.1. Sampling sites, study design, and sample collection

Characteristics for the 19 studied watersheds are shown in Table 1. Samples were collected between April 2009 and June 2011. In grazed beef watersheds in IN, VA, and WI animal droppings continuously take place, and animals always had stream access, except for the VA site, where livestock had stream access during the first, but not the second (see below), sampling event. In the remaining watersheds, there were specific periods of mechanical waste application, typically prior to crop planting (March–May) or following crop harvest (October–December). There were specific exceptions, however, to manure applications in select cases. In two watersheds (KY poultry, PA swine), the expected waste application did not take place (Table 1) due to factors such as soil moisture conditions. In the AR poultry watershed, most waste was applied outside the basin during the study period.

Sampling personnel were in contact with local producers and obtained livestock waste samples at the time of mechanical application, or from droppings in e.g. pastures adjacent to the stream, at the time of sampling. Waste samples (composites of 6–10 subsamples) represented a single livestock producer or a composite of multiple producers, as appropriate to each watershed. Livestock waste samples were collected as they were being applied to the field, or from waste left in the field if animals were pastured, or, if necessary, from the waste storage structure (e.g., lagoon, compost pile). In select cases where a small number of livestock of a different type were present in the watershed (Table 1), waste samples from these other animal types were also collected. Manure samples and most stream water and sediment