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Original Contribution

Antibiotic-Resistant Genes and Pathogens Shed by Wild Deer Correlate with Land Application of Residuals

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Abstract: The purpose of this study was to investigate genetic biomarkers of zoonotic enteric pathogens and antibiotic-resistant genes (ARGs) in the feces of white-tailed deer (*Odocoileus virginianus*) as related to proximity of deer to land that receives livestock manure or human waste biosolid fertilizers. Deer feces were collected in the St. Lawrence River Valley and Adirondack State Park of New York. *Campylobacter* spp. 16S rDNA was detected in 12 of 232 fecal samples (8 of 33 sites). *Salmonellae* were cultivated from 2 of 182 fecal samples (2 of 29 sites). Genetic virulence markers for Shiga-like toxin I (stx_1) and enterohemolysin (*hylA*) were each detected in one isolate of *Escherichia coli*; *E. coli* O157 was not detected in any of 295 fecal samples. ARGs detected in deer feces included *ermB* (erythromycin-resistant gene; 9 of 295 fecal samples, 5 of 38 sites), *vanA* (vancomycin-resistant gene; 93 of 284 samples, 33 of 38 sites), *tetQ* (tetracycline-resistant gene; 93 of 295 samples, 25 of 38 sites), and *sul(I)* (sulfonamide-resistant gene; 113 of 292 samples, 28 of 38 sites). Genetic markers of pathogens and ARGs in deer feces were spatially associated with collection near concentrated animal feeding operations (CAFOs; *Campylobacter* spp., *tetQ*, and *ermB*) and land-applied biosolids (*tetQ*). These results indicate that contact with human waste biosolids or animal manure may be an important method of pathogen and ARG transmission and that deer in proximity to land-applied manure and human waste biosolids pose increased risk to nearby produce and water quality.

Keywords: Concentrated animal feeding operations, Biosolids, Gastrointestinal pathogens, Antibiotic resistance genes, White-tailed deer, Sentinels

Introduction and Purpose

The emergence, spread, persistence, and evolution of infectious disease in wild animals are an inherently spatial process, embedded in complex landscapes, and influenced by human interactions both directly, and mediated through

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the environment (Becker et al. 2015). Proximity to human activities influences carriage of zoonotic pathogens and antimicrobial resistance (AMR) in wild animals (Alonso et al. 2016, 2017; Stedt et al. 2014; Bonnedahl et al. 2009; Rwego et al. 2008). Acquisition of zoonotic pathogens by wildlife through interactions with anthropogenic waste streams can negatively impact wildlife and threaten the biosecurity of water resources, produce grown for human consumption, and nearby livestock through subsequent interactions (Hsu et al. 2017; Singh et al. 2015; Porrero et al. 2014; Laidler et al. 2013).

Important interactions between wildlife and anthropogenic waste streams occur in agroecosystems. In the USA, livestock animals produce 335 million dry tons of manure annually (USEPA 2013), biosolids from human wastewater treatment total 7 million dry tons each year (USEPA 1999; Gerba and Smith 2005). The primary disposition of manure and biosolids is application to agricultural land as a fertilizer for grasses and crops grown for uses other than human consumption. These land application practices present a potential pathway for dissemination of pathogenic microorganisms and AMR to the environment. Pathogen and vector attraction reduction requirements exist for biosolids prior to application (40 C.F.R. § 503.32 1993; see US EPA 1999, 2003). Similar requirements do not apply for manure application, even though manure may contain many zoonotic pathogens of concern (Himathongkham 1999; Stanley et al. 1998; US EPA 2005). Recently, the US Food and Drug Administration reported that sales of antibiotics reached 13.5 million kg for use in domestic animals and 3.3 million kg for treatment of human illness in 2011 (USFDA 2012; USFDA 2013). Selective pressures favoring AMR that can be shed in feces result from the use of antibiotics for the treatment of human or livestock disease and for growth promotion and prophylaxis in livestock. AMR in human medicine and agriculture is a growing global concern. Even so, there are no minimum quality recommendations for land application of manure or biosolids regarding antibiotics, antibiotic-resistant microorganisms, or antibiotic-resistant genes (ARGs).

Smith et al. (2014) point out that it is virtually impossible to contain pathogenic- and antibiotic-resistant microorganisms within healthcare or food animal production systems owing to movement of these organisms and associated mobile genetic elements between domestic, domiciliated, and wild environments. Natural environments may normally contain AMR (Allen 2008). However, discharge of livestock manures and biosolids waste materials into the environment can increase the abundance and diversity of pathogens and AMR, with associated risks of increased human and animal exposures (e.g., Tien et al. 2017; Jahne et al. 2016; Rahube et al. 2014).

Management options for reducing the spread of pathogens and AMR via environmental pathways have been characterized, although effectiveness in regard to AMR is less well known than for pathogens (e.g., US EPA 2005; Pruden et al. 2013). Of note, wild animals may circumvent these management practices when they graze upon or otherwise frequent manure or biosolids-fertilized lands, nearby water impacted by their runoff, or water impacted by discharge from other human waste treatment systems (Furness et al. 2017; Allen et al. 2011; Porrero et al. 2014; Cole 2005; Renter et al. 2003). Associated risks to humans, wild animals, and domesticated animals are known, but not well characterized. For example, wild animals have been implicated as a risk of transmission of *Mycobacterium avium* subsp. *paratuberculosis* from infected livestock to non-infected livestock in nearby disease-free farms (Corn et al. 2005; Florou et al. 2008). Furness et al. (2017) reported that free-living small mammals were useful bioindicators of variation and distribution of antimicrobial resistance in the environment and potential for transmission from the environment to mammalian hosts, including humans. Singh et al. (2015) reported interspecies transmission of Shiga toxin-producing *Escherichia coli* between wild white-tailed deer and dairy cows in Michigan, highlighting the importance of wildlife in pathogen shedding dynamics and persistence in the environment and cowherds. Similar dynamics were reported between sheep, beef cattle, and wild red deer in Spain (Sánchez et al. 2010). Dias et al. (2015) screened *E. coli* isolates from wild red deer, roe deer, and boar in Portugal, and reported 9% of isolates were resistant to tetracycline. They reported that the three antibiotics to which isolates of *E. coli* were most frequently resistant to in their study belonged to the three classes of compounds with the most sales in the Portuguese veterinary field. *Salmonella* was not detected in fecal samples from wild deer in their study. Dunn et al. (2004) were not able to establish a prominent role of wild deer as a reservoir for *E. coli* O157 for cattle or humans in Louisiana. However, interactions of wildlife with produce-growing areas have been linked to at least three outbreaks of *E. coli* O157:H7 caused by consumption of tainted produce in the USA and Canada, including two attributed to contamination by wild deer (Cody et al. 1999; Jay et al. 2007; Laidler et al. 2013). In other studies, elucidating of the direction of transfer has been

elusive. For example, Smith et al. (2014) reported 31% prevalence of AMR *E. coli* in feces of wild hybrid deer in Ireland and posit that interaction of deer with livestock and farms may explain their presence, but cite a need for specific causal evidence to support their hypothesis.

This study compared distance from human waste biosolids-applied land, concentrated animal feeding operations (CAFOs), and non-CAFO livestock agriculture to the presence and concentration of enteric bacterial pathogens and ARGs in the feces of free ranging white-tailed deer (*Odocoileus virginianus*). Pathogens selected for study include *E. coli* O157:H7, *Campylobacter* spp., and *Salmonellae* owing their potential carriage by wild deer and importance to water and foodborne outbreaks of disease as reported by the Centers for Disease Control and Prevention (Alonso et al. 2017; Sasaki et al. 2013; Laidler et al. 2013). ARGs chosen for study include *tetQ* (tetracycline-resistant ribosomal protection protein), *ermB* (erythromycin-resistant 23S ribosomal RNA methyltransferase), *vanA* (vancomycin-resistant D-alanyl–D-lactate ligase), and *sul(I)* (sulfonamide-resistant dihydropteroate synthase) owing to their common detection in other reported wildlife sentinel studies, occurrence among bacteria in the human colon and intestines and rumens of cattle, and reported antibiotic use in the study area in human medicine and livestock agriculture (Hsu et al. 2017; Tien et al. 2017; Guerrero-Ramos et al. 2016; Alonso et al. 2016; Rahube et al. 2014; Munir and Xagoraraki 2011; McKinney et al. 2010; Anderson et al. 2008; Okamoto et al. 2001). The objectives of the study were to: (1) quantify the prevalence of ARGs and pathogens in white-tailed deer feces; (2) evaluate manure and human waste biosolid application to land as predictors of spatial patterns of ARGs and pathogens in the feces of white-tailed deer; and (3) identify wildlife mitigation practices used by local growers and operators to assess whether mitigation measures that limit pathogen and ARG spread by wildlife are needed.

Materials and Methods

Study Location

The study was conducted in the St. Lawrence River Valley (SLRV) and Adirondack foothills and mountains of New York State; 85% of sample sites were within St. Lawrence County (7300 km^2) and the remaining in three adjacent counties (Jefferson, Lewis, Franklin; entire study region = 19,800 km^2). The SLRV is a landscape of hayfields and pasturelands, row crops (predominantly maize), woodlots, abandoned farmland in various states of succession, and small villages. Interspersed throughout the valley are riparian corridors, state and county forest reserves, extensive wetlands, and wildlife management areas. This region supports a dense population of white-tailed deer (legal harvest $>$ 1 deer km^{-2}). In contrast, the Adirondack region is predominantly forested, and deer are less abundant (St. Lawrence County Board 2001; Jenkins and Keal 2004).

At the time of this study, there was 142,000 ha of farmland in St. Lawrence County; dairy agriculture dominated livestock production activities. Most farms were small family-operated dairies; according to USDA national agriculture statistics, 55% of dairy farms in 2012 had less than 50 cows (USDA 2007). These farms predominantly grazed their livestock; manure and bedding generated in barns were scraped and stored in piles or in manure storage pits until land application, predominantly on hayfields and maize crop fields cultivated by the dairy operators. In 2012, 122 farms totaling 196 ha produced fresh vegetables in St. Lawrence County; there were over 30 organic farming operations. Deer caused significant crop damage in the SLRV (St. Lawrence County Board 2001; Brown et al. 2004).

There has also been expansion of large dairy operations within St. Lawrence Country since the turn of the century; during the study period, dairy CAFOs comprised 4% of dairy farms, but contained 50% of the dairy cows in the county. Almost exclusively, dairy manures at CAFOs within the study region and study period were maintained in storage pits and anaerobic lagoons until application to land; one CAFO practiced anaerobic digestion of their manure prior to storage and land application. Liquid manures from storage pits, anaerobic lagoons, and anaerobic digester effluents were applied to land at agronomic rates to meet crop nutrient requirements, predominantly maize and various types of hay. Application practices were dominated by splash plate broadcast spreading (tankers pulled by tractors) at approximately 4 m above the ground surface. Occasionally, farmers practiced low-height splash plate spreading, drag hose spreading, or manure injection when near residential areas to reduce odor complaints. For the purposes of this work, we did not differentiate between manure application practices among CAFOs.

At the time of the study, municipal wastewater treatment was practiced in the large villages of the SLRV. These facilities produced class B biosolids that met requirements

for land application to non-produce crops. Biosolids were collected by contracted handlers and applied to land at agronomic rates to meet crop nutrient requirements. Septic systems were common in smaller villages and rural areas throughout the SLRV. Solids from these septic systems were hauled to local wastewater treatment plants for treatment and disposal. It is possible that some of these septic systems were substandard and may have contaminated nearby water bodies. Water quality has also reportedly been impaired by inadequate septic systems of some seasonal residences in the Adirondack Park (Terrie 2009). We did not survey septic systems within the area of study to determine their potential association to prevalence of ARGs or genetic pathogen biomarkers in the feces of white-tailed deer.

Bacteria and DNA Standards

DNA was sourced from the following organisms for DNA standards for endpoint PCR and qPCR described below: pNFD13-2 in *E. coli* S17-1 (MBA80; *tetQ*), *Enterococcus faecium* (ATCC 51559; *vanA*), *S. enterica* subsp. *enterica* ser. Typhimurium (ATCC 35664; *ttrRSBCA*; *sul(I)*), *Streptococcus pneumoniae* (ATCC BAA-1403; *ermB*), and *E. coli* O157:H7 (ATCC 43894; $rfbE_{O157:H7}$, *fliC*, *eaeA*, *hylA*, stx_1 and stx_2). Purified DNA from manufacturers included *Campylobacter jejuni* subsp. *jejuni* (ATCC 43446D-5) and *Oncorhynchus keta* (keta salmon) testes gDNA (#D-1626, Sigma-Aldrich).

Sample Collection

Deer feces were collected from 38 dispersed sites across the study location (see supplemental data file for coordinates of sample locations). Most sites were on state or county forest parcels; additional sites included six agricultural fields on the same property as a CAFO and two woodlots on local university campuses. At each sampling site, two 500-m transects were surveyed using standard methods for transect surveys of mammal sign (Wilson 1996). Transects were established where the land was consistent with preferred habitat for white-tailed deer, ran parallel to the border (within 10 m from the edge) of a forest parcel, and aligned with nearby agricultural land if present. Transects within each forest parcel were spaced a minimum of 1.6 km apart to reduce local spatial autocorrelation (Wilson 1996).

Fecal samples (approximately 30 g each) were collected from two different fecal droppings piles along each transect on every survey. The location of each sample was georeferenced (using a global positioning system). We aimed to sample only fresh deposits (moist, no obvious fungal growth); over time, ARG and manure pathogen concentrations may decline as feces decompose (Chee-Sanford et al. 2009; Rogers et al. 2011). Samples were collected into individual sterile 50-mL conical tubes by hand using sterile techniques and kept on ice while transported to the laboratory. Samples were maintained at 4°C during collection and transport and during storage in the laboratory prior to sample processing.

Sample Preparation

Each fecal sample was homogenized within 48 h of collection by mixing in their respective 50-mL conical tube using a sterile glass rod. Moisture contents were determined from 5-g aliquots gravimetrically following drying at 105°C for 24 h. Two 250-mg aliquots of each fresh homogenized sample were placed into a MoBio PowerSoil Bead tube for DNA extraction as described below. Additionally, two 250-mg aliquots were placed into sterile 2-mL tubes and archived at − 20°C.

Sample Enrichment

To improve detection of *Salmonella* spp. and *E. coli* O157:H7, selective enrichment and immunomagnetic separation were used to enrich their populations prior to endpoint PCR and qPCR. Briefly, 10 g of each fresh homogenized fecal sample was added to 90-mL buffered peptone water (BPW) in a Seward Circulator 400 filter bag (Seward, Bohemia, NY) and homogenized by stomaching in a Seward Stomacher 400 Circulator at 230 rpm for 45 s prior to being sealed shut and incubated at 35°C for 24 h. Following incubation, each stomacher bag was mixed by stomaching at 230 rpm for 45 s. *Salmonellae* and *E. coli* O157 were selected from 1.0-mL aliquots of this primary enrichment by immunomagnetic bead separation as per manufacturer's protocols (Dynal Dynabeads, Anti-Salmonella and Anti-O157, Invitrogen, Carlsbad, CA). Of note, secondary enrichment of *Salmonellae*-Anti-*Salmonella* bead complexes was in 10-mL Rapport Vasiladis Broth (RSV) at 35°C for 24 h rather than on selective agar.

Our study objective was detection of the presence of *Salmonella* spp. as indicated by the presence of the *ttrRSBCA* genetic marker (Malorny 2004); thus, isolation of typical colonies for further examination of virulence factors or species identification was not necessary. Secondary enrichment of *E. coli* O157-Anti-O157 bead complexes was on CHROMagar O157 for 24 h at 35°C. This facilitated selection of typical *E. coli* O157 isolates for genetic testing for $rfbE_{O157:H7}$ (Paton and Paton 1998), *fliC* (Gannon et al. 1997), *hylA* (Sanath Kumar et al. 2001) stx_1, stx_2, and *eaeA* (Ibekwe and Grieve 2003) to determine whether isolates were *E. coli* O157:H7 and to characterize the presence of virulence factors in each isolate. Following secondary enrichment, either 1 mL of *Salmonella* secondary enrichment broth or up to 10 typical isolates of *E. coli* O157 were each washed twice in 1 mL of phosphate-buffered saline (PBS; pH 7.2) with centrifugation at 6000×*g* and 4°C for 5 min between wash steps to pellet bacterial cells and then resuspended in 300-µL Qiagen Buffer AE (Qiagen, Valencia, CA) for DNA extraction.

DNA Extraction

DNA was extracted from fresh homogenized fecal samples, washed cell suspensions following bacterial enrichment, and from bacterial standards sourced from manufacturers. DNA from overnight cultures of bacteria standards (cultivated as directed by the respective commercial providers for each organism) was extracted using the MoBio Ultraclean Microbial DNA Isolation kit as per manufacturer's instructions (MoBio). Enriched *E. coli* O157:H7 and *Salmonella* spp. suspensions in Qiagen Buffer AE were boiled for 10 min and immediately quenched on ice; DNA was recovered with the supernatant following centrifugation at 6000×*g* at 4°C for 10 min. DNA was extracted from 250-mg aliquots of homogenized deer fecal samples using the MoBio PowerSoil DNA Extraction kit (MoBio, Carlsbad, CA) as per manufacturer's instructions. Cell lysis was by bead milling at 6.0 m s^{-1} for 30 s using the Fast Prep-24 Bead Beater (MP Biomedicals, Solon, OH). To quantify potential DNA loss during extraction from fecal material, a total of 50 replicate (control) deer fecal samples were spiked with 2-ng *O. keta* gDNA (between two and ten controls in each batch of 24 DNA extractions) and the *O. keta* ITS region 2 assay (SKETA) was used as an exogenous extraction and amplification control for qPCR as described previously (Haugland et al. 2005; Rogers et al. 2011). Average recovery of SKETA from these procedures was 43 ± 6%. Duplicate extraction blanks were processed with each batch of 24 fecal DNA extractions as a quality check for extraneous DNA introduced during laboratory procedures. All extracted DNA concentrations were measured using the Quant-iT dsDNA BR Assay kit (PicoGreen) with the Qubit fluorometer (Invitrogen; Carlsbad, CA) as per manufacturer's instructions. DNA extracts were stored at − 20°C until PCR analysis.

Endpoint PCR and qPCR

Primers and probes used in the study are listed in Table 1. Endpoint PCR was performed on a Thermo Electron Corporation PXE 0.2 thermal cycler for 45 cycles. Each 25-µL PCR consisted of 12.5 µL of 2X Applied Biosystems (Life Technologies, Thermo Fisher) AmpliTaq Gold PCR Master Mix, 12.5 pmol of each forward and reverse primer, 5.0-µL template DNA, and PCR-grade water. All reactions began with a hold at 95°C for 10 min to activate the hot-start enzyme, followed by 35–40 cycles of 15 s at 95°C, 30 s at the annealing temperature (Table 1) and 90 s at 72°C. All reactions ended with a hold at 72°C for 7 min. Reaction products were electrophoresed on precast 16 + 1, 2.2% agarose FlashGel DNA cassettes (Lonza, Basel Switzerland) with the FlashGel 50- to 1500-bp DNA marker and FlashGel Quantladder as per manufacturer's instructions. Electrophoresed DNA amplicons were viewed and documented with the FlashGel dock and FlashGel camera as per manufacturer's instructions. No template controls (NTCs) and positive controls (PCs) were included with each instrument run for quality control.

A Roche LightCycler 480 (Roche; Basal, Switzerland) was used for all qPCR. Reaction mixtures (25 µL) contained 12.5 µL of 2× LightCycler 480 Probe Master (Roche), 12.5-pmol forward and reverse primer, 5.0 pmol of fluorogenic probe, 5.0-µL template DNA, and 5.0-µL PCR-grade water. All reactions began with a hold at 95°C for 10 min followed by 45 cycles of 15 s at 95°C, 30 s at the annealing temperature, and either 30 s at the annealing temperature for assays with an annealing temperature of 60°C or greater, or 30 s at 72°C for assays with annealing temperatures less than 60°C (Table 1). Two NTCs and two PCs containing a known concentration of template DNA were included on each reaction plate. Standard curves were generated for each qPCR assay and used to measure gene copy numbers in each sample. Calibration curve equations and performance characteristics of the qPCR assays are reported in Supplemental Table 1. The range of quantifi-

Table 1. Endpoint PCR and qPCR Oligonucleotides and Amplification Conditions.

Target/locus	Primer and probe sequences (5′–3′)	Platform[a]	Anneal Temp[b] (°C)	References
Oncorhynchus keta ITS region 2	*f*:GGTTTCCGCAGCTGGG *r*:CCGAGCCGTCCTGGTCTA [6-FAM]-AGTCGCAGGCGGCC ACCGT-[NFQ-MGB]	A	60	Haugland et al. (2005)
Campylobacter spp. 16S rDNA	*f*:CACGTGCTACAATGGCATAT *r*:GGCTTCATGCTCTCGAGTT [6-FAM]-CAGAGAACAATCCGAAC TGGGACA-[NFQ-MGB]	A	58	Lund et al. (2004)
Salmonella spp. *ttrRSBCA*	*f*:CTCACCAGGAGATTACAACATGG *r*:AGCTCAGACCAAAAGTGACCATC [6-FAM]-CACCGACGGCGAGACC GACTTT-[NFQ-MGB]	A	65	Malorny et al. (2004)
E. coli O157:H7 $rfbE_{O157:H7}$	*f*:CGGACATCCATGTGATATGG *r*:TTGCCTATGTACAGCTAATCC	B	65–60[b]	Paton and Paton (1998)
E. coli O157:H7 *fliC*	*f*:CCGAATTCATGGCACAAGTCATTAATAC *r*:GTCATGGAAACCGTTGTCAC	B	65	Gannon et al. (1997)
Shiga-like toxin stx_1	*f*:GACTGCAAAGACGTATGTAGATTCG *r*:ATCTATCCCTCTGACATCAACTGC [6-FAM]-TGAATGTCATTCGC TCTGCAATAGGTACTC-[NFQ-MGB]	A	55	Ibekwe and Grieve (2003)
Shiga-like toxin stx_2	*f:ATTAACCACACCCCACCG* *r*:GTCATGGAAACCGTTGTCAC [TET]-CAGTTATTTTGCTGTGGA TATACGAGGGCT-[NFQ-MGB]	A	55	Ibekwe and Grieve (2003)
Intimin *eaeA*	*f*:GTAAGTTACACTATAAAAGCACCGTCG *r*:TCTGTGTGGATGGTAATAAATTTTTG [VIC]-AAATGGACATAGCATCAGCAT AATAGGCTTGCT-[NFQ-MGB]	A	55	Ibekwe and Grieve (2003)
Enterohemolysin A *hylA*	*f*:ACGATGTGGTTTATTCTGGA *r*:CTTCACGTGACCATACATAT	B	55	Sanath Kumar et al. (2001)
Tetracycline resistance *tetQ*	*f:AGGTGCTGAACCTTGTTTGATTC* *r:GGCCGGACGGAGGATTT* *[6-FAM]-TCGCATCAGCATCCCGCTC*-[NFQ-MGB]	A	60	Smith (2004)
Vancomycin resistance *vanA*	*f*:CTGTGAGGTCGGTTGTGCG *r*:TTTGGTCCACCTCGCCA *[6-FAM]-CAACTAACGCGGCACTG TTTCCCAAT*-[NFQ-MGB]	A	60	Volkmann et al. (2004)
Sulfonamide resistance *sul(I)*	*f*:CCGATATTGCTGAGGCGGACT *r*:AAGCTGAAGTCGGCGTTGG	B	55.9	Pei (2006)
Erythromycin resistance *ermB*	*f*:CTACAAGCGTACCTTGG *r*:TCTGGAACATCTGTGGTAT *[6-FAM]-TCATCCTAAACCAAAA GTAAACAGTGTC*-[NFQ-MGB]	A	60	Dela Cruz (2007)

[a]Platforms and master mix included: (A) qPCR: Roche LightCycler 480 with Roche Probe Master; (B) endpoint PCR: thermo electron corporation PXE 0.2 Thermal Cycler with Applied Biosystems AmpliTaq Gold PCR master mix.

[b]Anneal Temp = annealing temperature; annealing temperature for $rfbE_{O157:H7}$ began at 65°C for the first 10 cycles, decreasing to 60°C by cycle 15.

cation spanned the entire range of concentrations tested for all qPCR assays.

DNA extracts from fecal samples and from sample enrichments were analyzed by qPCR in duplicate. If the crossing point (C_p) of duplicates differed by less than one half cycle (0.5 C_p), the average C_p was used to calculate DNA copy number. A triplicate consensus reaction was performed if the C_p of duplicates differed by more than 0.5 C_p. In these cases, the triplicate reactions were examined. Any reaction that returned a $C_p > |0.5\ C_p|$ of the other two reactions was discarded, and the average C_p of remaining reactions used to calculate DNA copy number. Results from qPCR assays of gene markers in DNA extracted from enriched *E. coli* O157:H7 and *Salmonella* spp. (*ttrRSBCA*, stx_1, stx_2, and *eaeA*) were recorded as present/absent. The remaining qPCR assay results (*Campylobacter* 16S rDNA, *ermB*, *sul(I)*, *tetQ*, and *vanA*) were recorded quantitatively per gram dry weight of feces. Importantly, assays for pathogens and ARGs were independently performed. The presence of ARGs in a fecal sample does not imply association with any specific pathogen.

Data Analysis

The locations of CAFOs, non-CAFO livestock farms, and fields onto which human wastewater biosolids were applied to land within the study area were provided by the New York State Department of Environmental Conservation and were verified using high-resolution digital orthoimagery, ground-truthing, and direct inquiry of wastewater treatment plant operators who contract for the land application of their biosolids. Fields onto which human waste biosolids were applied were targeted because only three wastewater treatment plants used this practice in the study area. For practical purposes, locations of livestock farms and CAFOs were used as spatial predictors of manure application to land. It is important to note that it is not uncommon for large CAFOs to contract land for manure application. Although the frequency of manure application drops rapidly with distance from the CAFO of origin owing to costs associated with manure hauling, we have observed manure hauling distances as great as 15 km from CAFOs in the SLRV.

ArcGIS (ESRI Corp., Redlands CA USA) was used to determine the distance between each sample site and nearest potential source of ARG or pathogen (CAFO, non-CAFO animal agriculture operation, or biosolids application). The median distance of a sampling site to the nearest animal agriculture site (farm) was 7.5 km (range 0–44.7), CAFO was 9.8 km (0–46.5), and biosolids application site was 25.4 km (3.8–106.7).

One-tailed Fisher's exact tests were used to statistically evaluate whether the presence of the four ARGs and two genetic pathogen biomarkers was positively associated with proximity to each of three putative sources (CAFOs, non-CAFO livestock agriculture, biosolid applications). We used three distance classifications for proximity: 2, 6, and 10 km, based on distances white-tailed deer move on a daily, seasonal, or annual basis in our region (Tierson et al. 1985; Stewart et al. 2011; DeYoung and Miller 2011; Quinn et al. 2013). This resulted in 47 statistical tests ($6 \times 3 \times 3$ tests, minus seven for which there was no data). It remains controversial how best to make inferences from multiple tests: whether to apply the highly conservative Bonferroni criterion, in this case the critical $P = 0.001$, or else use the conventional criterion $P = 0.05$ (Gotelli and Ellison 2012). In this paper, we follow Gotelli and Ellison (2012) and report the P value, discuss inferences based on both the conservative Bonferroni criterion and the liberal conventional criterion, and caveat emptor; the major conclusions are unchanged.

While we report univariate tests, multiple logistic regressions (predictors: log distance to CAFO, non-CAFO livestock, biosolid application; outcome: marker presence/absence) were also done, with qualitatively similar results. However, model diagnostics indicated unacceptable violations of statistical assumptions that were probably due to the proportionately low number of detections and collinearity caused by a correlation in distance between biosolids application sites and (non-CAFO) livestock agriculture (distance biosolids—livestock agriculture $r_p = 0.71$, CAFO—livestock agriculture $r_p = 0.22$, CAFO—biosolids $r_p = 0.04$).

Farmer and Veterinarian Interviews

Interviews with CAFO operators, small-scale dairy operators, and produce growers were used to gain a greater understanding of farmer attitudes and practices with regard to deer management and antibiotic use. A local livestock veterinarian was interviewed to understand local on- and off-label use of antibiotics. The farm types included in the interviews were: two CAFO operators (medium and large operations), two organic farms (one dairy (non-CAFO grazer) and one produce), and one conventional produce grower. Interviews were recorded and transcribed and

consisted of brief open-ended discussions using the interview guide approach (Patton 2014). Farming operations ranging from total confinement conventional operations to 100% organic pasture operations were included to ensure an accurate cross section of practices and attitudes regarding deer control. For a dairy operation to be certified organic, pasture must be managed in a way that prevents erosion or water quality problems (i.e., fenced riparian buffer zones). Livestock over 6 months of age must have daily access to the outdoors. During the growing season, there must be daily access to managed pasture with edible forage. The use of antibiotics or other synthetic substances is prohibited; animals must be removed from the herd if antibiotics or synthetic substances are used. In regard to produce operations, organic certification by the Northeast Organic Farming Association of New York requires a buffer zone from any adjacent conventional operations. Not all non-CAFO farms produce organically in the SLRV.

Results

Presence and Prevalence of ARGs and Pathogens in Deer Feces

Summary results of our study are reported in Table 2; the full data set is available in the supplemental information. The presence of ARGs in deer feces was high: three ARGs (*tetQ*, *vanA,* and *sul(I)*) were each detected in approximately one-third of samples and at two-thirds of sites. The fourth, *ermB*, was detected in only 3.1% of samples, but at 13.2% of sites. At least one of these four ARGs was detected at every site. Although our work reflects detection of ARGs from whole fecal samples, our results compare well to other recent studies that report 6.5–31% prevalence of resistance to various antimicrobials, including those of our study, in *E. coli* isolated from feces of wild deer in Ireland, Spain, and Portugal (Dias et al. 2015; Alonso et al. 2016; Smith et al. 2014).

Genetic biomarkers for *Campylobacter* spp. (16S rDNA) and *Salmonella* spp. (*ttrRSBCA*) were detected in 5.2 and 1.1% of samples, respectively, but at 24.2 and 6.9% of sites. *E. coli* O157:H7 was not detected in any deer fecal samples (Table 2); however, one isolate of *E. coli* was positive for both stx_1 and *hylA*. Shiga-like toxin genes were not detected in any other deer fecal samples. Although prior studies with wastewater have suggested widespread presence of enterohemolysin genes in environmental isolates of *E. coli* (Boczek et al. 2006), *hylA* was detected in only one deer fecal sample in this study. Detections of at least one potential pathogen occurred at 26.3% of sites. In comparison with these study results, Branham (2005) reported a 7.7% presence of *Salmonella*, but did not detect *E. coli* O157:H7 in hunter-harvested deer in Texas. Other studies have reported prevalence of *E. coli* O157 in fecal specimens from wild white-tailed deer including 0.4% (n = 226) in Louisiana, 2.4% (n = 212) in Kansas, and 0.25% (n = 1608) in Nebraska (Dunn et al. 2004; Sargeant et al. 1999; Renter et al. 2001). Singh et al. (2015) reported 1, 6, and 22% prevalence of Shiga toxin-producing *E. coli*, enterohemorrhagic *E. coli*, and enteropathogenic *E. coli*, respectively, in deer fecal samples (n = 74) in Michigan, as well as greater shedding in June versus March.

Prevalence in Relation to Distances to Putative Sources

Summary results are presented in Table 3. Owing that *E. coli* O157:H7 was not detected in any samples, it was excluded. Furthermore, samples collected within 2 km of CAFOs were not tested for *ttrRSBCA*, and there were no samples collected within 2 km of biosolids application sites.

Proximity of the sampling site to CAFO operations and biosolids applications sites were predictors for the detection of *tetQ* (prevalence = 45.2%, presence = 31.5%), suggesting that CAFOs and land-applied biosolids may be a source of *tetQ* in deer within the study region. This was true even when a (conservative) Bonferroni correction was applied to account for multiple (47) tests. From the conventional criterion (P = 0.05), proximity of sampling site to CAFO operations was also a predictor of *ermB* (prevalence = 20.9%, presence = 3.1%) and *Campylobacter* 16S rDNA (prevalence = 16.8%; presence = 5.2%) in deer feces, suggesting that CAFOs may also be a source of *ermB* and *Campylobacter* in deer within the study region. In contrast, *sul(I)* (prevalence = 55.7%, presence = 38.7%) and *vanA* (prevalence = 46.9%, presence = 32.7%) were frequently detected, but widely distributed. Considering, we could not correlate increased prevalence of *sul(I)* or *vanA* in deer feces to proximity to any one of the three putative sources. Although the genetic marker for *Salmonella* spp. was very infrequently detected (*ttrRSBCA*; prevalence = 11.3%, presence = 1.1%), its presence may be correlated with proximity to non-CAFO livestock agriculture sites.

Table 2. Summary Table of ARG and Pathogen Detections.

Marker	Samples					Sites				
	N	# Pos	% Pres	Mean Conc	Range	N_{Sites}	$N_{Samples}$	% Pres	% Prev	Mean ± SD Conc
Antibiotic resistance										
Tetracycline (*tetQ*)	295	93	31.5	1.1×10^5	6.1×10^2–1.7×10^8	38	8 (1–22)	65.8	45.2	$(1.3 \pm 4.5) \times 10^6$
Erythromycin (*ermB*)	295	9	3.1	2.1×10^5	6.5×10^3–6.4×10^7	38	8 (1–22)	13.2	20.9	$(3.4 \pm 7.1) \times 10^6$
Vancomycin (*vanA*)	284	93	32.7	3.8×10^4	6.5×10^2–8.1×10^6	38	7 (1–22)	86.8	46.9	$(0.9 \pm 1.4) \times 10^5$
Sulfonamide (*sul(I)*)	292	113	38.7	NA	NA	38	8 (1–22)	73.7	55.7	NA
Pathogens										
Campylobacter (16S rDNA)	232	12	5.2	6.6×10^5	1.8×10^5–1.5×10^8	33	7 (1–21)	24.2	16.8	$(1.0 \pm 1.8) \times 10^6$
Salmonella (*ttrRSBCA*)	182	2	1.1	NA	NA	29	6 (2–12)	6.9	11.3	NA
Typical *E. coli* O157[a]	306	27	8.8	NA	NA	38	8 (1–22)	34.2	29.6	NA
O157 antigen ($rfbE_{O157:H7}$)		0	0.0	NA	NA			0.0	0.0	NA
Shiga-like toxin I (stx_1)		1	0.3	NA	NA			2.6	12.5	NA
Shiga-like toxin II (stx_2)		0	0.0	NA	NA			0.0	0.0	NA
Enterohemolysin (*hylA*)		1	1.0	NA	NA			2.6	12.5	NA
Intimin (*eaeA*)		0	0.0	NA	NA			0.0	0.0	NA
H7 flagellar antigen (*fliC*)		0	0.0	NA	NA			0.0	0.0	NA
stx_1 + *hylA*		1	0.3	NA	NA			2.6	12.5	NA
E. coli O157:H7[b]	306	0	0.0	NA	NA	38	8 (1–22)	0.0	0.0	NA

''Samples'' provides the attributes of the full sample set. ''# Pos'' indicates the number of samples in which the marker was detected. ''% Pres'' is the percent of all samples for which the marker was detected. ''Mean Conc'' is the Log-mean concentration (copies g^{-1}) of those samples for which the marker was detected; concentration was quantified for a subset of markers only. ''Range'' provides the extreme values.

''Sites'' summarizes the attributes per site across all sites. ''N_{Sites}'' is the number of sites, whereas ''$N_{Samples}$'' is the mean and range of sample sizes per site. ''% Pres'' is the percent of sites at which the marker was detected. ''% Prev'' is the mean percent of samples for which the marker was detected per site, including only those sites at which it was detected at least once. ''Mean ± SD Conc'' is the mean and standard deviation of the geometric mean concentrations (copies g^{-1}) of sites where the marker was detected; concentration was quantified for a subset of markers only.

NA not applicable; only presence/absence data are available.

[a]Bacteria exhibiting characteristics typical of *E. coli* O157 when plated onto CHROMagar O157 following primary enrichment and immunomagnetic separation that were selected for genetic biomarker testing.

[b]Isolates of typical *E. coli* O157 that tested positive for $rfbE_{O157:H7}$ and *fliC*.

Interviews

Both farmers and producers interviewed in this study expressed acceptance of wildlife, particularly white-tailed deer. Concerns focused primarily on consumption of crops or disturbance of fences designed to confine cows. Produce growers recognized the potential for contamination of crops from wildlife. Dairy farmers recognized wildlife as a potential source of disease for their livestock.

Despite the acceptance of wildlife as ''…part of nature'' and ''…part of farming'', every farmer surveyed took preventative action to minimize the impacts of wildlife, protect produce biosecurity, and preserve herd health. This was especially the case for produce operations. Despite the high cost of fencing, it was the most often described deterrent on produce operations; one organic produce grower who described the deer population as ''uncountable'' and ''thick as mosquitoes'' spent several thousand dollars for a 2.4-m tall fencing system. This small grower sought an option that ''didn't require constant vigilance.'' He considered dogs, but decided against it in fear that there were ''too many variables'' and that the abundant deer population could ''eat $20,000 [worth of crops] in two nights'' if given the chance. Another local grower determined that the combination of dogs and baited electric fencing was an effective control mechanism for her farm.

Dairy operators expressed fewer concerns over wildlife. Their defensive efforts were limited to either owning dogs or operating a total confinement system eliminating exposure of their cattle to wildlife. In fact, the dairy CAFO operators viewed the confinement of their herds, without access to pasture, as protection from contamination of their herds by browsing deer, and protection of deer from

Table 3. Summary Table of Relating Proximity to Predictor Variables (CAFOs, Non-CAFO Livestock Farms, and Biosolids Application Sites) to Antibiotic Resistance Gene Markers and Pathogen Gene Markers Detections.

Marker	Distance (km)	CAFO			Non-CAFO Livestock			Biosolids		
		Nearer	Further	*P*	Nearer	Further	*P*	Nearer	Further	*P*
Antibiotic resistance										
Tetracycline (*tetQ*)	≤ 2	90 (29)	25 (266)	< **0.001**	30 (27)	32 (218)	0.75	NA	NA	NA
	≤ 6	56 (106)	18 (189)	< **0.001**	33 (115)	31 (180)	0.35	72 (18)	29 (277)	< **0.001**
	≤ 10	46 (153)	18 (152)	< **0.001**	30 (153)	33 (142)	0.69	43 (30)	30 (265)	0.08
Erythromycin (*ermB*)	≤ 2	0 (29)	3 (266)	0.700	4 (27)	3 (268)	0.29	NA	NA	NA
	≤ 6	7 (106)	1 (189)	**0.006**	3 (115)	3 (180)	0.63	0 (18)	3 (277)	0.75
	≤ 10	6 (143)	1 (152)	**0.008**	5 (153)	1 (142)	0.02	0 (30)	3 (265)	0.70
Vancomycin (*vanA*)	≤ 2	21 (29)	33 (255)	0.75	11 (27)	35 (257)	0.99	NA	NA	NA
	≤ 6	29 (106)	35 (178)	0.72	27 (114)	36 (170)	0.94	17 (18)	34 (266)	0.92
	≤ 10	28 (143)	38 (141)	0.95	28 (150)	38 (134)	0.96	30 (30)	33 (254)	0.58
Sulfonamide (*sul(I)*)	≤ 2	0 (29)	43 (263)	1.00	26 (27)	40 (265)	0.89	NA	NA	NA
	≤ 6	15 (108)	53 (184)	1.00	37 (117)	40 (175)	0.73	17 (18)	40 (274)	0.96
	≤ 10	21 (145)	56 (147)	1.00	34 (153)	43 (139)	0.92	43 (30)	38 (262)	0.35
Pathogens										
Campylobacter (16S rDNA)	≤ 2	30 (10)	5 (222)	**0.01**	0 (10)	5 (222)	0.75	NA	NA	NA
	≤ 6	9 (56)	4 (176)	0.09	5 (82)	5 (150)	0.75	0 (16)	6 (216)	0.75
	≤ 10	6 (80)	5 (152)	0.38	4 (102)	6 (130)	0.72	0 (28)	6 (204)	0.81
Salmonella (*ttrRSBCA*)	≤ 2	NA	NA	NA	14 (7)	1 (175)	**0.04**	NA	NA	NA
	≤ 6	0 (25)	1 (157)	0.75	2 (66)	1 (116)	0.75	0 (4)	1 (178)	0.75
	≤ 10	0 (48)	1 (134)	0.75	1 (85)	1 (97)	0.75	0 (15)	1 (167)	0.75

Reported for each distance classification and predictor variable are the percent of samples positive for the biomarker of interest and sample size (*n*). Fisher's exact test was used to test the hypothesis that a difference in the percent detection of the gene marker of interest existed between samples taken closer than, or further away from, the distance classification of interest for each predictor variable. *P* values ≤ 0.05 are highlighted; the Bonferroni-corrected critical *P* value < 0.001.

NA not applicable; data not available within 2 km of CAFO, non-CAFO livestock agriculture, or biosolids application sites for the gene of interest.

potential contamination from livestock. One of the CAFO operators stated that local dairy farmers that grazed their cattle in pastures were more susceptible to cross-contamination than confinement dairy operations. In addition, farmers who practiced total confinement described their herd health programs as effective for maintaining a healthy herd, obtaining maximum milk yields, and limiting penalties associated with contaminated milk. Financial penalties associated with milk contaminated with antibiotics were reported to limit the use of antibiotics in dairy operations. However, farmers described using ceftiofur hydrochloride therapeutically for the treatment of foot rot and bovine respiratory disease (BRD), and Rumensin® (Monensin, USP; Elanco Animal Health, Greenfield, IN) and Bovatec® (Zoetis, Parsippany, NJ) as growth promoters in the feed. Rumensin® is effective for prevention and control of *Eimeria bovis* and *Eimeria zuernii*, the two most common parasites that cause coccidiosis in dairy cows. The veterinarian reported that tetracycline was a common additive in medicated milk replacers for dairy calves and that sulfaquinoxaline (a sulfonamide drug) was common for treatment of coccidiosis in affected animals.

A recurrent theme expressed by all in the study was the apparent increase in the size of the local deer population over the past half-century and the effects this has had on local ecosystems. Farmers attributed the increase to a variety of factors including mild winters, less hunting of deer due to cultural shifts and stricter regulations, and increased hunting of coyote. Most farmers hunted, leased their land out for hunting, and obtained doe permits. Despite the number of farmers who hunted with doe permits as a control mechanism, farmers were unsure of the effectiveness of hunting to control deer populations.

Discussion

In this study, the presence of *tetQ*, *sul(I)*, *ermB*, and *vanA* in deer feces ranged from 3 to 39%, and they were detected at 13–87% of sample sites. Both *vanA* and *sul(I)* were detected frequently in deer feces in the SLRV and were distributed widely. Much of the valley is rural, and residents rely heavily on septic systems, some of which may leak and contaminate water in the region. Additionally, approximately 130,000 year round residents and 200,000 seasonal residents live in the Adirondack State Park; 7–10 million people visit the park each year (Jenkins and Keal 2004). The majority of these residents and visitors also depend on septic systems (Terrie 2009). Of note, vancomycin is banned for use in animal agriculture in the USA. Sulfonamides are used in both human and veterinary medicine, but generally more so in human medicine as a therapeutic agent (Pei 2006). Although extensive use of these antibiotics for treatment of infections in dairy cows was reported by Heur et al. (2009), the local veterinarian interviewed in this study described the use of sulfonamides in livestock in the SLRV to be limited (only used as coccidiostats for calves and heifers).

Proximity of a sampling site to dairy CAFOs was a predictor of prevalence of *Campylobacter* spp., *tetQ*, and *ermB* in deer feces; concentrations of *Campylobacter* spp. 16S rDNA in deer feces were also greater in proximity to CAFOs. Detection of *tetQ* in deer feces in proximity to livestock farms and CAFOs is consistent with the regional popularity of tetracyclines as a medicated milk replacer for calves. A local veterinarian described the use of tetracyclines and neomycin in livestock in the study area as "extensive." Several researchers have recognized increased odds, up to 5 times, of detecting tetracycline resistance from farm sources over natural areas (Kozak et al. 2008; Livermore 2001). Tetracycline is subject to widespread use; it is often used as a first line antimicrobial for disease prevention and growth promotion in food animals, likely contributing to the high rates of resistance associated with tetracycline use in agriculture (Kozak et al. 2008). Although farmers and the veterinarian interviewed in this study did not report use of erythromycin, formulations are available for the treatment of mastitis in cows and often have the advantage of a short withholding time for milk. Of note, many dairy producers manage their own antibiotic use in the region of study, and there were no regulatory reporting requirements for antibiotic use by livestock operators in the USA during the period of study.

Within the SLRV and during the period of study, wastewater treatment plants in the villages of Potsdam, Colton, and Oswegatchie contracted for the land application of their human waste biosolids. Application of biosolids occurred within 10 km of sample collection at three of 38 study locations. From this limited data set, a significantly greater prevalence of *tetQ* in deer feces was detected in proximity to biosolids application. Increased detection of this ARG is almost certainly attributable to human use of tetracycline and inadequate removal of *tetQ* from biosolids waste streams during conventional wastewater treatment (Pruden et al. 2013; Li et al. 2017). Although tetracycline resistance genes in dairy manure have been reported to be 1–2 orders of magnitude greater than that in human waste biosolids, these ARGs are also known to be highly abundant in human wastewater biosolids residuals (5.5×10^6–4.3×10^9 copies g^{-1}; Brooks et al. 2007; Munir and Xagoraraki 2011; Munir et al. 2011). Indeed, their presence in biosolids can be expected to be approximately 1000 times greater than in treated wastewater effluents (Munir et al. 2011).

The increased prevalence of *tetQ* and *ermB* in deer feces in proximity to manure and biosolids applications reported in this study is consistent with several geographically distributed studies (e.g., Africa, Europe, North America) that have evidenced proximity to human activities influences the antibiotic susceptibility profiles of the gut bacteria of various wild animals, including deer (e.g., Rolland et al. 1985; Rwego et al. 2008; Mariano et al. 2008; Bonnedahl et al. 2009; Allen et al. 2011; Jobbins and Alexander 2015; Singh et al. 2015; Furness et al. 2017). The potential for wild animals to serve as sentinel species or reservoirs for spread of AMR has been documented on scales ranging from local (small mammals, e.g., Allen et al. 2011; Furness et al. 2017), to regional (cervids, boars, and birds, e.g., Dias et al. 2015; Poeta 2009; Bonnedahl et al. 2009; Stedt et al. 2014), to global, including dissemination of AMR to polar regions by wild birds (e.g., Hernández et al. 2012; Sjölund et al. 2008). Increased prevalence of *Campylobacter* in deer feces in proximity to CAFOs in this study is consistent with earlier reports regarding transmission of enteropathogenic and Shiga toxin-producing *E. coli* between dairy cows and wild white-tailed deer in shared agroecosystems in Michigan (Singh et al. 2015).

Following an extensive review of the literature, Greig et al. (2015) concluded that there was general acceptance that contact with sewage or animal manure could be an important method of global dissemination of resistance

genes, yet the picture was confusing considering the intricacies of environmental and anthropogenic factors. For example, Sapkota (2007) documented greater concentrations of enterococci and elevated percentages of antibiotic-resistant enterococci in water downstream of mid-Atlantic swine CAFOs as compared to upstream of the CAFO. Pei (2006) reported similar results with increases in ARGs in river sediments corresponding to increased agricultural and human activity. Mariano et al. (2008) demonstrated that impala in South Africa drinking from rivers contaminated with tetracycline-resistant *E. coli* were nearly 20 times more likely to shed tetracycline-resistant *E. coli* in their feces than unexposed impala. Although it is clear there is a relationship between land application sites for biosolids and dairy manures and increased carriage of *Campylobacter*, *tetQ*, and *ermB* in wild deer, we cannot establish a definitive transmission pathway, whether a result of direct contact with manure or biosolids residuals or contact following further dissemination into the environment. Our study was also limited by our inability to directly sample all land-applied dairy manures and biosolids in the area of study during the study period; thus, we do not have access to definitive initial concentrations of *Campylobacter*, *tetQ* and *ermB* in these residuals during the period of study. However, we have previously measured and reported *Campylobacter* concentrations in dairy manures sourced from anaerobic lagoons, manure storage pits, and anaerobic digesters at selected CAFOs in the study region in other studies we have conducted during the period of this study. We have previously documented its prevalence in these source materials (Narula et al. 2011; Jahne et al. 2015; Jahne et al. 2016). Further, it is important to point out that land application of manure and biosolids residuals is designed to limit runoff to nearby rivers or streams through the use of management practices such as grassed and riparian buffers, setback distances, and land application rates, among other practices that would favor the importance of a transmission pathway involving direct contact with residuals on the landscape over secondary contact with subsequently contaminated water. Tetracycline ARGs in both dairy manures and biosolids residuals have been demonstrated to persist over background soil concentrations for more than 4 months following manure or biosolids application (Munir and Xagoraraki 2011). We have demonstrated in our own work persistence of *Campylobacter* in manure amended soils at temperatures ranging from sub-zero to 35°C for up to 4 months (Rogers et al. 2011). This persistence provides significant opportunities for subsequent transmission to deer that transit and forage upon manure- or biosolids-fertilized grass or crop fields. During the period of study, we visually observed, and on a limited basis documented via game cameras, deer in the St. Lawrence River Valley transiting and foraging in crop and hay fields within days to weeks following land application of dairy manures and biosolids.

White-tailed deer are abundant within the agricultural St. Lawrence River Valley lowlands of New York and are responsible for significant crop damage (St. Lawrence County Agricultural and Farmland Protection Board 2001; Jenkins and Keal 2004; Brown et al. 2004). Increased prevalence and concentrations of *Campylobacter* spp. and ARGs in the feces of wild white-tailed deer were spatially correlated with CAFOs and land-applied biosolids. Interactions of deer with biosolids and CAFO manures may influence carriage rates, presenting increased risk through subsequent interactions to the biosecurity of livestock, wild game meat, and produce grown in the St. Lawrence River Valley. *Campylobacter* is one of the top five pathogens contributing to bacterial foodborne illness resulting in hospitalizations in the USA and Canada (Thomas et al. 2013; Scallan et al. 2011). Protecting produce crops from contamination by fecal pathogens is a concern for growers. Standard mitigation practices (e.g., grass and riparian buffers) are designed to limit runoff of fecal pathogens and ARGs from land-applied residuals to produce. Transmission of ARGs and manure pathogens by white-tailed deer from land-applied residuals to produce is a concern because deer can negate these remediation measures. One commonly used barrier to prevent deer intrusion into produce-growing areas, as indicated by produce growers in this study, is fencing. While fencing can be an effective barrier to exclude deer, it must be properly installed and maintained for it to be effective. It is not uncommon for a white-tailed deer to crawl under or jump fences up to 2.4 m in height (Halls 1984). Additional measures have been proposed and implemented across the country, including physical separation of CAFOs by a mile or more from produce fields (US FDA 2008). Considering the migratory range of deer and results of this study, buffer sizes may need to be larger and mitigation measures more restrictive to prevent transmission of pathogenic microorganisms and antibiotic resistance genes from land-applied residuals to produce.

Conclusions

This study investigated the presence, concentration, and distribution of antibiotic resistance genes and zoonotic pathogens in the feces of white-tailed deer as related to the proximity to land that received livestock manure and human waste biosolid fertilizers. Their prevalence and concentration in the feces of deer were spatially correlated with these activities, suggesting that CAFO manures and biosolids are likely sources for deer, although we cannot rule out all other potential sources such as contaminated water within the focus habitats. This finding is in contrast to farmer-held views that confining their dairy herds virtually eliminates the potential for cross-contamination of deer populations and dairy cows; there may be benefit to dairy cow herd health, but it appears the use of antibiotics in herd health programs results in manure residues applied to cropland which the white-tailed deer come into contact with or ingest while feeding. Similarly, additional control measures to improve biosolids quality may be needed to reduce prevalence of ARGs in biosolids prior to land application, thereby reducing transmission to wild animals. White-tailed deer can move quickly over large distances, spreading diseases into undesirable places such as recreational waters or produce-growing areas. Further research exploring the influence of specific interactions of deer with land-applied residuals on disease prevalence and improved deer trajectory modeling may help to elucidate specific risks associated with these activities.

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References

Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J (2008) Call of the wild: antibiotic resistance genes in natural environments, Nature Reviews Microbiology 8:251–259; https://doi.org/10.1038/nrmicro2312 [Online March 1, 2010]

Allen SE, Boerlin P, Janecko N, Lumsden JS, Barker IK, Pearl DL, Reid-Smith RJ, Jardine C (2011) Antimicrobial resistance in generic *Escherichia coli* isolates from wild small mammals living in swine farm, residential, landfill, and natural environments in southern Ontario, Canada, Applied and Environmental Microbiology 77:882–888; https://doi.org/10.1128/aem.01111-10 [Online December 3, 2010]

Alonso CA, González-Barrio, D, Tenorio C, Ruiz-Fons F, Torres C (2016) Antimicrobial resistance in faecal *Escherichia coli* isolates from farmed red deer and wild small mammals. Detection of a multiresistant *E. coli* producing extended-spectrum beta-lactamase, Comparative Immunology, Microbiology and Infectious Diseases 45:34–39; https://doi.org/10.1016/j.cimid.2016.02.003 [Online February 18, 2016]

Alonso CA, Mora A, Díaz D, Blanco M, González-Barrio D, Ruiz-Fons F, Simón C, Blanco J, Torres C (2017) Occurrence and characterization of *stx* and/or *eae*-positive *Escherichia coli* isolated from wildlife, including a typical EPEC strain from a wild boar, Veterinary Microbiology 207:69–73; https://doi.org/10.1016/j.vetmic.2017.05.028 [online June 1, 2017]

Anderson JF, Parrish TD, Akhtar M, Zurek L, Hirt H (2008) Antibiotic resistance of Enterococci in American Bison (*Bison bison*) from a nature preserve compared to that of Enterococci in pastured cattle, Applied and Environmental Microbiology 74(6):1726–1730; https://doi.org/10.1128/aem.02164-07 [Online February 1, 2008]

Becker DJ, Streicker DG, Altizer S (2015) Linking anthropogenic resources to wildlife–pathogen dynamics: a review and meta-analysis, Ecology Letters 18(5):483–95; https://doi.org/10.1111/ele.12428 [Online March 21, 2015]

Boczek LA, Johnson CH, Rice EW, Kinkle BK (2006) The widespread occurrence of the enterohemolysin gene *hylA* among environmental strains of *Escherichia coli*, FEMS Microbiology Letters 254:281–284; https://doi.org/10.1111/j.1574-6968.2005.00035.x [Online January 1, 2006]

Branham LA, Carr MA, Scott CB, Callaway TR (2005) *E. coli* O157 and *Salmonella* spp. in white-tailed deer and livestock, Current Issues in Intestinal Microbiology 6(2):25–29.

Brooks JP, Maxwell SL, Rensing C, Gerba CP, Pepper IL (2007) Occurrence of antibiotic-resistant bacteria and endotoxin associated with the land application of biosolids, Canadian Journal of Microbiology 53:616–622; https://doi.org/10.1139/W07-021 [Online June 22, 2007]

Brown TL, Decker DJ, Curtis PD (2004) Farmers' estimates of economic damage from white-tailed deer in New York State. Human Dimensions Research Unit, Series 04-3. Department of Natural Resources, Cornell University, Ithaca, NY.

Bonnedahl J, Drobni M, Gauthier-Clerc M, Hernandez J, Granholm S, Kayser Y, Melhus A, Kahlmeter G, Waldenström J, Johansson A, Olsen B (2009) Dissemination of Escherichia coli with CTX-M Type ESBL between humans and yellow-legged gulls in the South of France, Plos One, 4:e5958; https://doi.org/10.1371/journal.pone.0005958 [Online June 18, 2009]

Chee-Sanford J, Mackie R, Koike S, Lin Y, Yannarell A, Maxwell S, Aminov R (2009) Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste, Journal of Environmental Quality, 38(3):1086–1108; https://doi.org/10.2134/jeq2008.0128 [Online May, 2009]

Cody SH, Glynn MK, Farrar JA, Cairns KL, Griffin PM, Kobayashi, J, Fyfe M, Hoffman R, King AS, Lewis JH, Swaminathan B, Bryant RG, Vugia DG (1999) An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice, Annals of Internal Medicine 130(3):202–209; https://doi.org/10.7326/0003-4819-130-3-199902020-00005 [Online February 2, 1999]

Cole D, Drum DJ, Stallknecht DE, White DG, Lee MD, Ayers S, Sobsey M. Maurer JJ (2005) Free-living Canada geese and antimicrobial resistance, Emerging Infectious Diseases 11(6):935–938; https://doi.org/10.3201/eid1106.040717 [Online June, 2005]

Corn JL, Manning EJB, Sreevatsan S, Fischer JR (2005) Isolation of *Mycobacterium avium* subsp. Paratuberculosis from free-ranging birds and mammals on livestock premises, Applied and Environmental Microbiology 71(11):6963–6967; https://doi.org/10.1128/aem.71.11.6963-6967.2005 [Online November, 2005]

Dela Cruz WP, Richardson JY, Broestler JM, Thornton JA, Danaher PJ (2007) Rapid determination of macrolide and lincosamide resistance in group B *Streptococcus* isolated from vaginal-rectal swabs, Infectious Diseases in Obstetrics and Gynecology Vol. 2007, Article ID 46581, 6 pages, https://doi.org/10.1155/2007/46581

DeYoung RW, Miller KV (2011) White-tailed deer behavior. In: Hewitt DJ (ed) Biology and management of white-tailed deer. CRC Press, Boca Raton, Florida, USA, pp. 311–351.

Dias D, Torres RT, Kronvall G, Fonseca C, Mendo S, Caetano T (2015) Assessment of antibiotic resistance of *Escherichia coli* isolates and screening of *Salmonella* spp. in wild ungulates from Portugal, Research in Microbiology 166:584–593; https://doi.org/10.1016/j.resmic.2015.03.006 [Online April 11, 2015]

Dunn JR, Keen JE, Moreland D, Thompson RA (2004) Prevalence of *Escherichia coli* O157:H7 in white-tailed deer from Louisiana, Journal of Wildlife Diseases 40(2):361–365; https://doi.org/10.7589/0090-3558-40.2.361 [Online February, 2004]

Florou M, Leontides L, Kostoulas P, Billinis C, Sofia M, Kyriazakia I, Lykotrafitis F (2008) Isolation of *Mycobacterium avium* subspecies Paratuberculosis from non-ruminant wildlife living in the sheds and on the pastures of Greek sheep and goats, Epidemiology and Infection 136:644–652; https://doi.org/10.1017/s095026880700893x [Online June 20, 2007]

Furness LE, Campbell A, Zhang L, Gaze WH, McDonald RA (2017) Wild small mammals as sentinels for the environmental transmission of antimicrobial resistance, Environmental Research 154:28–34; https://doi.org/10.1016/j.envres.2016.12.014 [Online December 22, 2016]

Gannon VP, D'Souza S, Graham T, King RK, Rahn K, Read S (1997) Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic *Escherichia coli* strains, Journal of Clinical Microbiology 35(3):656–662.

Gerba CP, Smith JE Jr (2005) Sources of pathogenic microorganisms and their fate during land application of wastes, Journal of Environmental Quality 34(1):42–48; https://doi.org/10.2134/jeq2005.0042 [Online January 2005]

Gotelli NJ, Ellison AM (2012) A primer of ecological statistics, 2nd edition, Sinauer Associates, Sunderland, MA.

Greig J, Rajić A, Young I, Mascarenhas M, Waddell L, LeJeune J (2015) A scoping review of the role of wildlife in the transmission of bacterial pathogens and antimicrobial resistance to the food chain, Zoonoses and Public Health 62(4):269–284; https://doi.org/10.1111/zph.12147 [Online August 30, 2014]

Guerrero-Ramos E, Cordero J, Molina-González D, Poeta P, Igrejas G, Alonso-Calleja C, Capita R (2016) Antimicrobial resistance and virulence genes in enterococci from wild game meat in Spain, Food Microbiology 53:156–164; https://doi.org/10.1016/j.fm.2015.09.007 [Online September 11, 2015]

Halls LK (1984) White-tailed deer: ecology and management, Stackpole, Harrisburg, PA.

Haugland RA, Siefring SC, Wymer LJ, Brenner KP, Dufour AP (2005) Comparison of *Enterococcus* measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis, Water Research 39:559–568; https://doi.org/10.1016/j.watres.2004.11.011 [Online December 24, 2004]

Heur H, Kopmann C, Bihn C, Top E, Smalla K (2009) Spreading antibiotic resistance through spread manure: characteristics of a novel plasmid type with low % G + C content. Environmental Microbiology 11(4):937–949; https://doi.org/10.1111/j.1462-2920.2008.01819.x. [Online December 3, 2008]

Hernández J, Stedt J, Bonnedahl J, Molin Y, Drobni M, Calisto-Ulloa N, Gomez-Fuentes C, Astorga-España MS, González-Acuña D, Waldenström J, Blomqvist M, Olsen B (2012) Human-associated Extended Spectrum β-Lactamase (ESBL) in the Antarctic. Applied and Environmental Microbiology 76:2056–2058; https://doi.org/10.1128/aem.07320-11 [Online January 13, 2012]

Himathongkham S, Bahari S, Riemann H, Cliver D (1999) Survival of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in cow manure and cow manure slurry, FEMS Microbiology Letters 178(2):251–257. https://doi.org/10.1111/j.1574-6968.1999.tb08684.x [Online September, 1999]

Hsu TT, Mitsch WJ, Martin, JF. Lee J (2017) Towards sustainable protection of public health: The role of an urban wetland as a frontline safeguard of pathogen and antibiotic resistance spread, Ecological Engineering 108(B):547–555; https://doi.org/10.1016/j.ecoleng.2017.02.051 [Online March 6, 2017]

Ibekwe AM, Grieve CM (2003) Detection and quantification of *Escherichia coli* O157: H7 in environmental samples by real-time PCR, Journal of Applied Microbiology 94(3), 421–431; https://doi.org/10.1046/j.1365-2672.2003.01848.x [Online February 12, 2003]

Jahne MA, Rogers SW, Holsen TM, Grimberg SJ, Ramler IP (2015) Emission and dispersion of bioaerosols from dairy manure application sites: human health risk assessment, Environmental Science and Technology 49(16):9842–9849; https://doi.org/10.1021/acs.est.5b01981 [Online July 9, 2015]

Jahne MA, Rogers SW, Holsen TM, Grimberg SJ, Ramler IP, Kim S (2016) Bioaerosol deposition to food crops near manure application: quantitative microbial risk assessment, Journal of Environmental Quality 45(2):666–674; https://doi.org/10.2134/jeq2015.04.0187 [Online February 12, 2016]

Jay MT, Cooley M, Carychao D, Wiscomb GW, Sweitzer RA, Crawford-Miksza L, Farrer JA, Lau DK, O'Connell J, Millington A, Asmudson RV, Atwill, ER, Mandrell RE (2007) *Escherichia coli* O157:H7 in feral swine near spinach fields and cattle, central California coast, Emerging Infectious Diseases 13(12):1908–1911; https://dx.doi.org/10.3201/eid1312.070763 [Online December, 2007]

Jenkins T, Keal A (2004) The Adirondack Atlas, Syracuse University Press, Syracuse, NY.

Jobbins SE, Alexander KA (2015) Whence they came – antibiotic-resistant *Escherichia coli* in African wildlife, Journal of Wildlife Diseases 51(4):811–820; https://doi.org/10.7589/2014-11-257 [Online July 29, 2015]

Kozak GK, Boerlin P, Janecko, N (2008) Antimicrobial resistance in *Escherichia coli* isolates from swine and wild small mammals in the proximity of swine farms and in natural environments in Ontario, Canada, Applied and Environmental Microbiology 75(3):559–566; https://doi.org/10.1128/aem.01821-08 [Online December 1, 2008]

Laidler MR, Tourdjman M, Buser GL, Hostetler T, Repp KK, Leman R, Samadpour M, Keene WE (2013) *Escherichia coli* O157:H7 infections associated with consumption of locally grown strawberries contaminated by deer, Clinical Infectious Diseases 57(8):1129–1134; https://doi.org/10.1093/cid/cit468 [Online July 21, 2013]

Li N, Sheng GP, Lu YZ, Zeng RJ, Yu HQ (2017) Removal of antibiotic resistance genes from wastewater treatment plant effluent by coagulation, Water Research 111:204–212; https://doi.org/10.1016/j.watres.2017.01.010 [Online January 4, 2017]

Livermore D, Warner M, Hall L, Enne V, Projan S, Dunman P, Wooster S, Harrison G (2001) Antibiotic resistance in bacteria from magpies (*Pica pica*) and rabbits (*Oryctolagus cuniculus*) from west Wales, Environmental Microbiology 3(10):658–661; https://doi.org/10.1046/j.1462-2920.2001.00239.x [Online October, 2001]

Lund M, Nordentoft S, Pedersen K, Madsen M (2004) Detection of *Campylobacter* spp. in chicken fecal samples by real-time PCR, Journal of Clinical Microbiology 42:5125–5132; https://doi.org/10.1128/jcm.42.11.5125-5132.2004 [Online November, 2004]

Malorny B, Paccassoni E, Fach P, Bunge C, Martin A, Helmuth R (2004) Diagnostic real-time PCR for detection of *Salmonella* in food. Applied and Environmental Microbiology 70(12):7046–7052; https://doi.org/10.1128/aem.70.12.7046-7052.2004 [online July 12, 2004]

Mariano V, McCrindle C, Picard, J (2008) Case- control study to determine whether river water can spread tetracycline resistance to unexposed impala (*Aepyceros melampus*) in Kruger National Park (South Africa). Applied Environmental Microbiology 75(1):113–118; https://doi.org/10.1128/aem.01808-08 [Online October 31, 2008]

McKinney C, Loftin KA, Meyer MT, Davis JG, Pruden A (2010) tet and sul antibiotic resistance genes in livestock lagoons of various operation type, configuration, and antibiotic occurrence, Environmental Science and Technology 44(16):6102–6109; https://doi.org/10.1021/es9038165 [Online July 21, 2010]

Munir M, Xagoraraki I (2011) Levels of antibiotic resistance genes in manure, biosolids, and fertilized soil, Journal of Environmental Quality 40:248–255; https://doi.org/10.2134/jeq2010.0209

Munir M, Wong K, Xagoraraki I (2011) Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan, Water Research 45:681–693; https://doi.org/10.1016/j.watres.2010.08.033 [Online August 27, 2010]

Narula R, Grimberg SJ, Rogers S, Mondal S (2011) Pathogen reduction and factors responsible for pathogen reduction in dairy farm operations treating dairy manure, Biological Engineering Transactions 4(3):115–131; https://doi.org/10.13031/2013.39813 [Online April, 2011]

Okamoto M, Takano K, Maeda N (2001) Distribution of the tetracycline resistance determinant tetQ gene in oral isolates of black-pigmented anaerobes in Japan, Molecular Oral Microbiology 16(4):224–228; https://doi.org/10.1034/j.1399-302x.2001.160405.x [Online August 2001]

Paton JC, Paton AW (1998) Detection and characterization of toxigenic *Escherichia coli* by using multiplex PCR assays for *stx1*, *stx2*, *eaeA*, enterohemorrhagic *E. coli hylA*, *rfb*O111 and *rfb*O157, Journal of Clinical Microbiology 36(2):598–602.

Patton MQ (2014) Qualitative research and evaluation methods: integrating theory and practice, Sage Publications, Thousand Oaks, CA.

Pei R, Kim SC, Carlson K, Pruden A (2006) Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG), Water Research 40(12):2427–2435; https://doi.org/10.1016/j.watres.2006.04.017 [Online June 6, 2006]

Poeta P, Radhouani H, Pinto L, Martinho A, Rego V, Rodrigues R, Goncalves A, Rodrigues J, Estrepa V, Torres C, Igrejas G (2009) Wild boars as reservoirs of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* of different phylogenetic groups, Journal of Basic Microbiology 49:584–588; https://doi.org/10.1002/jobm.200900066 [Online October 6, 2009]

Porrero MC, Harrison E, Fernández-Garayzábal JF, Paterson GK, Díez-Guerrier A, Holmes MA, Domínguez L (2014) Detection of *mecC*-Methicillin-resistant *Staphylococcus aureus* isolates in river water: a potential role for water in the environmental dissemination, Environmental Microbiology Reports 6:705–708; https://doi.org/10.1111/1758-2229.12191 [Online July 14, 2014]

Pruden A, Joakim Larsson DG, Amezquita A, Collignon P, Brandt KK, Graham DW, Lazorchak JM, Suzuki S, Silley P, Snape JR, Topp E, Zhang T, Zhu YG (2013) Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment, Environmental Health Perspectives 121:878–885; https://doi.org/10.1289/ehp.1206446 [Online August, 2013]

Quinn ACD, Williams DM, Porter WF (2013) Landscape structure influences space use by white-tailed deer, Journal of Mammalogy 94(2), 398–407; https://doi.org/10.1644/11-MAMM-A-221.1 [Online April 16, 2016]

Rahube TO, Marti R, Scott A, Tien YC, Murray R, Sabourin L, Zhang Y, Duenk P, Lapen DR, Topp E (2014) Impact of fertilizing with raw or anaerobically digested sewage sludge on the abundance of antibiotic-resistant coliforms, antibiotic resistance genes, and pathogenic bacteria in soil and on vegetables at harvest, Applied and Environmental Microbiology 80(22):6698–6907; https://doi.org/10.1128/aem.02389-14 [Online August 29, 2014]

Renter DG, Sargeant JM, Hygenstrom SE, Hoffman JD, Gillispie JR (2001) *Escherichia coli* O157:H7 in free-ranging deer in Nebraska, Journal of Wildlife Diseases 37:755–760; https://doi.org/10.7589/0090-3558-37.4.755

Renter DG, Sargeant JM, Oberst RD, Samadpour M (2003). Diversity, frequency, and persistence of *Escherichia coli* O157 strains from range cattle environments. Applied and Environmental Microbiology 69(1):542–547; https://doi.org/10.1128/aem.69.1.542-547.2003; https://doi.org/10.1128/aem.69.1.542-547.2003

Rogers SW, Donnelly M, Peed L, Kelty CA; Mondal S, Zhong Z, Shanks OC (2011) Decay of bacterial pathogens, fecal indicators, and real-time quantitative PCR genetic markers in manure-amended soils. Applied and Environmental Microbiology 77(14):4839–4848; https://doi.org/10.1128/aem.02427-10 [Online June 3, 2011]

Rolland RM, Hausfater G, Marshall B, Levy SB (1985) Antibiotic-resistant bacteria in wild primates: increased prevalence in baboons feeding on human refuse, Applied and Environmental Microbiology 49(4):791–794.

Rwego IB, Isabirye-Basuta G, Gillespie TR, Goldberg TL (2008) Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda. Conservation Biology 22(6):1600–1607; https://doi.org/10.1111/j.1523-1739.2008.01018.x [Online August 13, 2008]

Sanath Kumar H, Otta SK, Karunasagar I (2001) Detection of Shiga-toxigenic *Escherichia coli* (STEC) in fresh seafood and meat marketed in Mangalore, India by PCR, Letters in Applied Microbiology 33(5):334–338; https://doi.org/10.1046/j.1472-765x.2001.01007.x [Online November 6, 2001]

Sánchez S, Martínez R, Rey J, García A, Blanco J, Blanco M, Blanco JE, Mora A, Herrera-León S, Echeita A, Alonso JM (2010) Pheno-genotypic characterisation of *Escherichia coli* O157:H7 isolates from domestic and wild ruminants, Veterinary Microbiology 142(3–4):445–449; https://doi.org/10.1016/j.vetmic.2009.10.009 [Online October 23, 2009]

Sapkota A, Curriero F, Gibson K, Schwabb K (2007) Antibiotic-resistant enterococci and fecal indicators in surface water and groundwater impacted by a concentrated swine feeding operation, Environmental Health Perspectives 115(7):1040–1045; https://doi.org/10.1289/ehp.9770 [Online March 22, 2011]

Sargeant JM, Hafer DJ, Gillispie JR, Oberst RD, Flood SJ (1999) Prevalence of *Escherichia coli* O157:H7 in white-tailed deer sharing rangeland with cattle, Journal of the American Veterinary Medical Association 215:792–794.

Sasaki Y, Goshima T, Mori T, Murakami M, Haruna M, Ito K, Yamada Y (2013) Prevalence and antimicrobial susceptibility of foodborne bacteria in wild boars (*Sus scrofa*) and wild deer (*Cervus Nippon*) in Japan, Foodborne Pathogens and Disease 10(11):985–991; https://doi.org/10.1089/fpd.2013.1548 [Online November, 2013]

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM (2011) Foodborne illness acquired in the United States-major pathogens. Emerging Infectious Diseases 17(1):7–15; https://doi.org/10.3201/eid1701.p11101 [Online January, 2011]

Singh P, Sha Q, Lacher DW, Del Valle J, Mosci RE, Moore JA, Scribner KT, Manning SD (2015) Characterization of enteropathogenic and Shiga toxin-producing *Escherichia coli* in cattle and deer in a shared agroecosystem, Frontiers in Cellular and Infection Microbiology 5:29; https://doi.org/10.3389/fcimb.2015.00029 [Online April 1, 2015]

Sjölund M, Bonnedahl J, Hernandez J, Bengtsson S, Cederbrant G, Pinhassi J, Kahlmeter G, Olsen B (2008) Dissemination of multidrug-resistant bacteria into the arctic, Emerging Infectious Diseases 14:70–72; https://doi.org/10.3201/eid1401.070704 [Online January, 2008]

Smith MS, Yang RK, Knapp CW, Niu Y, Peak N, Hanfelt MM, Galland JC, Graham DW (2004) Quantification of tetracycline resistance genes in feedlot lagoons by real-time PCR. Applied and Environmental Microbiology 70(12):7372–7377; https://doi.org/10.1128/aem.70.12.7372-7377.2004 [Online November 28, 2004]

Smith S, Wang J, Fanning S, McMahon BJ (2014) Antimicrobial resistant bacteria in wild mammals and birds: a coincidence or cause for concern? Irish Veterinary Journal 67:8; https://doi.org/10.1186/2046-0481-67-8 [Online April 25, 2014]

St. Lawrence County Agricultural Development Plan; St. Lawrence County Agricultural and Farmland Protection Board; St. Lawrence County Board of Legislators: Canton, NY, 2001; http://www.co.st-lawrence.ny.us/slcpo. [Accessed November 4, 2017]

Stanley KN, Wallace JS, Currie J, Diggle P, Jones K (1998) The seasonal variation of thermophilic campylobacters in cattle. Journal of Applied Microbiology 85:472–480; https://doi.org/10.1046/j.1365-2672.1998.853511.x [Online September, 1998]

Stedt J, Bonnedahl J, Hernandez J, McMahon BJ, Hasan B, Olsen B, Drobni M, Waldenström J (2014) Antibiotic resistance patterns in *Escherichia coli* from gulls in nine European countries. Infection Ecology and Epidemiology 4: https://doi.org/10.3402/iee.v4.21565. http://doi.org/10.3402/iee.v4.21565; https://doi.org/10.3402/iee.v4.21565 [Online January 10, 2014]

Stewart KM, Bowyer TR, Weisberg P.J (2011) White-tailed deer behavior. In: Hewitt DJ (ed) Biology and management of white-tailed deer. CRC Press, Boca Raton, Florida, USA, pp. 181–217.

Terrie PG (2009) Compromise, continuity, and crisis in the Adirondack Park – Shortcomings in environmental protection. In (W.F. Porter, J.D. Erickson, R.S. Whaley eds.) The Great Experiment in Conservation – Voices from the Adirondack Park, Syracuse University Press, Syracuse, NY.

Tien YC, Li B, Zhang T, Scott A, Murray R, Sabourin L, Marti R, Topp E (2017) 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, Science of the Total Environment 581–582:32–39; https://doi.org/10.1016/j.scitotenv.2016.12.138 [Online March 1, 2017]

Thomas MK, Murray R, Flockhart L, Pintar K, Pollari F, Fazil A, Nesbitt A and Marshall B (2013) Estimates of the burden of foodborne illness in Canada for 30 specified pathogens and unspecified agents, circa 2006. Foodborne Pathogens and Disease 10(7):639–648; https://doi.org/10.1089/fpd.2012.1389 [Online May 9, 2013]

Tierson WC, Mattfeld GF, Sage Jr. RW, Behrend DF (1985). Seasonal movements and home ranges of white-tailed deer in the Adirondacks. The Journal of Wildlife Management 49(3):760–769; https://doi.org/10.2307/3801708 [Online October 23, 2007]

U.S. Department of Agriculture (2007) Census of Agriculture; United States Department of Agriculture –National Agricultural Statistics Service; Washington, DC, 2007.

U.S. Environmental Protection Agency (1999) Biosolids generation, use, and disposal in the United States, United States Environmental Protection Agency, Municipal and Industrial Solid Waste Division, Office of Solid Waste, EPA530-R-99-009, September, 1999.

U.S. Environmental Protection Agency (2003) Control of pathogens and vector attraction in sewage sludge (including domestic septage), under 40 CFR Part 503; EPA/625/R92/013,USEPA: Cincinnati, OH, 2003.

U.S. Environmental Protection Agency (2005) Detecting and mitigating the environmental impact of fecal pathogens originating from confined animal feeding operations: review; EPA/600/R-06/021; Rogers, S. and. Haines, J. USEPA: Cincinnati, OH, 2005.

U.S. Environmental Protection Agency (2013) Literature review of contaminants in livestock and poultry manure and implications for water quality; EPA/820/R-12/002; USEPA, Office of Water: Washington, D.C., 2013.

U.S. Food and Drug Administration (2008) Guide to minimize microbial food safety hazards for fresh fruits and vegetables; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington DC, October, 2008.

U.S. Food and Drug Administration (2012) Estimates of antibacterial drug sales in human medicine. U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Office of Surveillance and Epidemiology, Silver Spring, MD.

U.S. Food and Drug Administration (2013) 2011 Summary report on antimicrobials sold or distributed for use in food-producing

animals. U.S. Food and Drug Administration, Center for Veterinary Medicine, Rockville, MD.

Volkmann H, Schwartz T, Bischoff P, Kirchen S and Obst U (2004) Detection of clinically relevant antibiotic-resistance genes in municipal wastewater using real-time PCR (TaqMan). Journal of Microbiological Methods 56(2):277–286. DOI: https://doi.org/10.1016/j.mimet.2003.10.014 [online October, 2003]

Wilson DE, Cole ER, Nichols JD, Rudran R, Foster MS (1996) Measuring and monitoring biodiversity – Standard methods for mammals. Smithsonian Institution Press, Washington, D.C.