Cornell University
College of Veterinary Medicine

USDA

Pathogens in Rural and Agricultural Water and Watersheds 2010

State of Knowledge and Future Directions

October 2010

Cover Photograph

Sheldrake Point Vineyards

2006

Evan Yokum

Cornell University
College of Veterinary Medicine

USDA

Pathogens in Rural and Agricultural Water and Watersheds 2010

State of Knowledge and Future Directions

October 2010

Table of Contents

Foreword

This synthesis report was prepared based on the findings of the *National Synthesis Workshop: Pathogens (Bacteria, Viruses, and Protozoa) in Rural and Agricultural Watersheds*. The workshop, sponsored by the United States Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA)[1], was held at Cornell University May 17-20, 2010 and organized by Dr. Dwight D. Bowman.[2] Participants consisted of many current and former recipients of NIFA Agriculture and Food Research Initiative[3] grants, as well as other experts in various fields from academia, industry, and government. This report, compiled by Dr. Ellen Carlin, a veterinarian, summarizes findings from those NIFA studies and places them in the context of expertise put forth at the conference. Information on grant-funded research was obtained from the USDA's National Water Program Current Research Information System,[4] a compilation of summaries and updates from grant recipients. Because many of the grant-funded studies are still in progress, the results cited may be preliminary; these limitations are acknowledged where applicable. For some studies, the published papers were numerous; a select number were chosen for inclusion in the text, but a complete listing is contained in the Appendix. Where challenges and areas for further study were identified, they are emphasized as "Gaps" throughout the report and listed in the Appendix.

A draft of this report was circulated among all workshop participants and other grantees whose work was cited, and their comments were incorporated into the final publication to the greatest extent possible. Not all participants are presumed to agree with every conclusion of this report, but collectively, their input led to its findings.

---

[1] Formerly Cooperative State Research, Education, and Extension Service (CSREES).

[2] Bowman DD. Current knowledge of sources, fate and transport of rural and agricultural watershed pathogens: a national synthesis workshop. Contract no.: 2009-65102-05842.

[3] Formerly National Research Initiative.

[4] United States Department of Agriculture Web site. National Water Program - Grants Awarded by the NIFA Agriculture and Food Research Initiative (Formerly NRI): Water and Watershed competitive grants**.** Available at http://www.usawaterquality.org/watershed/projects/default.html

Acknowledgements

All workshop participants contributed to the writing of this report, either via the ideas they presented at the workshop, or later as the paper was developed and they were asked to write or edit sections. Many provided extensive content or edits. In a very real sense, all are authors. Participation is also gratefully acknowledged for many of those grant recipients whose work is summarized in the report, but who were unable to attend the workshop. Their enthusiasm for the project and their insights into the content added significant value. Brian Richards of Cornell University provided a thoughtful and thorough review of the manuscript in its final stages. Mary Linton, Janice Liotta, and Araceli Lucio-Forster, also of Cornell, are thanked for their invaluable assistance preparing for the workshop, and making it a success.

Abstract

In response to a request for application from the United States Department of Agriculture (USDA), Cornell University proposed a workshop to address the question of what is known about the sources, transport, and fate of rural and agricultural watershed pathogens. The workshop was held in May 2010 at the Cornell University College of Veterinary Medicine in Ithaca, NY. Through lectures and breakout sessions, participants offered their knowledge and debated questions about what the most important pathogens are, where they come from, how they move, how to effectively regulate them, and, importantly, what remains unknown. This report delineates risks and reduction parameters that will be useful for both regulators and educators to protect this water, and contains viable solutions for reducing and managing pathogen loading in the environment. The group developed a list of those viruses, bacteria, and protozoa that they deemed most important to the discussion of rural and agricultural watersheds and human health. Proposals for further research studies and education are also included.

Keywords: watershed, pathogen, feces, manure, viruses, bacteria, protozoa, fungi

List of Figures and Tables

List of Acronyms

AFO	animal feeding operation
AFRI	Agriculture and Food Research Initiative
BCV	bovine coronavirus
BEV	bovine enterovirus
BF	base flow conditions
BRV	bovine rotavirus
bST	bovine somatotropin
BMP	best management practice
BOD	biological oxygen demand
CAFO	confined animal feeding operation
CDC	Centers for Disease Control and Prevention
CWA	Clean Water Act
CWS	constructed wetland systems
DLVO	Derjaguin-Landau-Verwey-Overbeek theory
EPA/U.S. EPA	United States Environmental Protection Agency
FIB	fecal indicator bacteria
GIS	geographic information system
GPS	global positioning system
HEV	hepatitis E virus
HSA	hydrologically sensitive area
IMS	immunomagnetic separation
MPN	most probable number
MST	microbial source tracking
NIFA	National Institute of Food and Agriculture
NRCS	Natural Resources Conservation Service
NRI	National Research Initiative
PCR	polymerase chain reaction
PFGE	pulsed field gel electrophoresis
qPCR	quantitative (real-time) polymerase chain reaction
rbST	recombinant bovine somatotropin
rep-PCR	repetitive extragenic palindromic polymerase chain reaction
RT-PCR	reverse transcription polymerase chain reaction
SDS	sodium dodecyl sulfate
SDWA	Safe Drinking Water Act
SRO	storm runoff events
STEC	shiga toxin-producing *E. coli*
USDA	United States Department of Agriculture
USGS	United States Geological Survey
UV	ultraviolet

Executive Summary

In response to a request for application from the United States Department of Agriculture (USDA), Cornell University proposed a workshop to address the question of what is known about the sources, transport, and fate of rural and agricultural watershed pathogens. The workshop was held in May 2010 at the Cornell University College of Veterinary Medicine in Ithaca, NY. Through lectures and breakout sessions, participants offered their knowledge and debated questions about what the most important pathogens are, where they come from, how they move, how they can be effectively regulated, and, importantly, what remains unknown.

Much is known about the microbiology of human pathogens and how they sometimes end up in water supplies. The USDA has funded a tremendous body of work on this issue. We recognize the value of all of this work, as well as that funded by other agencies like the U.S. Environmental Protection Agency and the National Institutes of Health. It was our specific charge to succinctly relate the findings of that research as it relates to rural and agricultural watersheds, and to determine where further resources should be targeted.

The fact is, we know a lot about the pathogens that thrive in animal waste. We understand much about their biology, how they are transported, and how waste can be treated to remove them or at least to minimize their numbers. So while we present many knowledge gaps and recommendations for research in this report, it is with the intention that practical measures will still be taken in the present based on the knowledge that is currently available to us.

This report delineates risks associated with pathogens in rural and agricultural watersheds, and strategies to reduce these risks. This information will be useful for both regulators and educators to protect these watersheds, and contains viable solutions for reducing and managing pathogen loading in the environment. Covered topics include an overview of pathogens in the watershed; survival; transport and fate; risk assessment and modeling; remediation and mitigation; and the path forward, given what we know and what we do not know. All of these topics were covered in considerable detail, primarily by outlining the work done in these areas by NIFA grant recipients, but also addressing other research where relevant. Key findings in each area were:

Pathogens and the watershed: Watersheds are susceptible to myriad land-based inputs of bacteria, viruses, and protozoa, which are all known pathogenic contaminants of watersheds. In order to prevent their spread and mitigate their potential impacts, we must forge a better understanding of pathogen dynamics, including sources (i.e., human or other animal), post-shed behavior, zoonotic capabilities, and how environmental factors affect pathogen fate. We must also develop and assess better indicators for human and other animal sources of feces, particularly those that can distinguish between human and other animal sources. The table below (presented again in Chapter 1 as Table 2) represents a list of those viruses, bacteria, and protozoa that deemed by the workshop panel as most important to the discussion of rural and agricultural watersheds and human health. The list was deliberately kept concise. A secondary list of pathogens of potential concern was also compiled (Table 3). No helminths were found to be of sufficient importance for either list. The only fungus represented is Microsporidia, on the secondary list.

Pathogens of importance

ORGANISM	TYPICAL SOURCE	NOTES
Viruses		
Enteroviruses	Human feces*	
Hepatitis A	Human feces*	
Hepatitis E	Swine feces	Sporadic in people in the U.S.; infections more common in developing countries
Myxovirus	Humans, Vertebrates	Mainly respiratory
Norovirus	Human feces*	Animal sources under investigation
Rotavirus	Human feces*	Animal sources under investigation
Bacteria		
Campylobacter jejuni	Poultry feces	
E. coli (enterohemorrhagic, diarrheagenic, shiga-toxin producing); enteropathogenic (EPEC); enteroinvasive (EIEC); and enteroaggregative (EAgg EC)	Cattle feces	
Salmonella enterica (serotypes Newport, Typhimurium, Enteritidis, Montevideo)	Cattle, poultry, and swine feces	
Protozoa		
Cryptosporidium		
C. hominis	Human feces*	No animal reservoir
C. parvum	Calf feces	Primarily calves less

		than 30 days old
Giardia duodenalis		
Assemblage A (I and II)	Human feces* and that of other mammals	AII only in people
Assemblage B	Human feces*	No animal reservoir

*Could enter rural and agricultural watershed through septic systems or municipal wastewater

Survival and reservoirs: We do not yet understand the survival potentials of all pathogens of concern under all scenarios, such as variabilities in weather/climate, hydrology, and land use. Nor do we have a complete understanding of survival dynamics in niches where pathogens survive until their further transmission. We must better understand pathogen stability and viability in manure, soil, and water under different environmental treatment and disinfection conditions. Better models for the prediction of pathogen survival are needed. Knowledge is also limited in terms of pathogen growth and survival in environmental microhabitats. Development of improved mathematical descriptions of growth, survival, and retention for modeling pathogen behavior at the field and watershed scales would be of value in ultimately mitigating pathogen spread.

Transport and fate: Substantial progress has been made in identifying component processes that control waterborne pathogen transmission, but we still do not have the capability to predict how combinations of processes control overall migration of pathogens through watersheds. We can currently predict mean behavior fairly well, but not extreme release events or fate of low concentrations of infectious pathogens that are likely to produce outbreaks. Transport processes are complex and the various scales in which they are studied (e.g., behavior of single pathogens, pore scale, column scale, field scale) remain fragmented. Many factors play a role, and many questions remain unanswered. Transport remains an excellent area for further study but, due to the cross-cutting nature of the work, there must be better collaboration between transport researchers and microbiologists at the very least, and, ideally, including epidemiologists, producers, and others who may contribute relevant information and analysis. Lastly, microbial source tracking research has made considerable progress, but effective tools that differentiate pathogens among farm animals and from humans and wildlife are not yet available.

Sampling, isolation, identification, and monitoring: These processes are means to an end, but they must be employed judiciously, not only because it is economically unfeasible to test all waters, but it is also impractical and probably unnecessary. For the most part, assessing specific pathogens entering the source water of a surface water drinking water treatment plant might not provide large quantities of valuable information. At this time, it is difficult to foresee how ground water drinking water supplies could be routinely monitored for most manure-associated pathogens, except perhaps using certain agents as indicators of potential contamination. Key initiatives should be to agree upon standardized methods, which should be developed only for the detection of those water contaminant pathogens considered to be of particular importance; develop real time assay systems or field kits that yield results for indicators within two to six hours; develop indicators for viruses and protozoan parasites in water; and **e**valuate the variability in sensitivity due to different inhibitors in various samples for source tracking.

Risk assessment and modeling: Predicting the occurrence of individual outbreaks might never be possible. Even though we lack detailed predictive, site-based models, the current knowledge base readily supports development general vulnerability maps for pathogen dissemination based on four to five well known factors that tend to lead to higher risk. Ultimately, we probably will be unable to go past probabilistic models, but we should be able to identify major controls on critical outcomes and high likelihood of disease outbreaks. Prediction is currently limited by a lack of understanding of interaction of different processes (sources, transmission, survival, etc.) in complex environmental systems. Process-based exposure and risk models could provide a good framework for this type of prediction.

Remediation and mitigation: In most cases, we remain unable to differentiate the source of a pathogen, and without that ability, we cannot determine the proportional contribution of any one group (humans, livestock, wildlife). It is, therefore, a challenge to recommend mitigatory practices without a real understanding of underlying risk that would necessitate such practices. Nevertheless, the use of multiple barriers is a necessary step to preventing transmission of pathogens from farms. To truly be of value, their efficacy must be assessed. Where possible, bacteria, viruses, and protozoa should be assessed, although where that is financially not feasible, low-cost indicators like fecal coliforms could be used. Instating a tracking and response system for large producers could prove to be of considerable value. The practicality of instating farm-side testing must be carefully considered, however, particularly in light of the fact that other entities, such as wastewater treatment plants, are not required to monitor for pathogens, either. The benefits/effectiveness of natural and manmade barriers and multi-barrier approaches, and innovative technologies that can effectively reduce pathogenic contaminants from farm-origin point and non-point sources, should be developed. Producers are encouraged to maintain adequate vegetative cover and minimizing congregation of cattle in streams. In general, the choice of pathogen control measures should be risk-based, and options presented to farmers should be cost effective.

The path forward: Best practices for manure management were discussed. Many agreed that recommended practices should be thought of as *beneficial* management practices (rather than *best)*, since what works for one farm in one set of circumstances may not be applicable to another. Recommendations were made for how extension agents and governments can work with farmers. A list of possible extension course topics was presented. Viable options for economic incentivization and farmer participation were presented, including scenarios such as group payments to farmers based on the overall quantity and quality of water flowing from their watershed. A set of possibilities for databases to collate information and allow for pattern recognition was also put forward. Finally, a list of prioritized research study recommendations was developed in the following areas:

1. Source, survival, and reservoirs
2. Transport and fate
3. Sampling, isolation, and identification
4. Risk assessment, modeling, and monitoring
5. Remediation and mitigation
6. Medicine and epidemiology
7. Management

Introduction

The United States goes to significant lengths to ensure that its drinking and recreational water supplies are safe and healthy. Public drinking water in the U.S. is closely monitored for contaminants and pathogens by the Environmental Protection Agency (U.S. EPA), state and municipal government bodies, and private water utilities. Some recreational waters are also monitored, but even unmonitored waters benefit collaterally from strict regulation of human, livestock, and industrial waste.

Nevertheless, outbreaks and sporadic cases of waterborne illness do occur, with reports of several thousand people affected per year. Actual case numbers are likely higher.

Since 2005, the United States Department of Agriculture (USDA) has funded research on potential pathogens and their relationship to the water supply. These studies were funded through the Cooperative State Research, Education, and Extension Service (CSREES) as National Research Initiative (NRI) grants, and later came under the umbrella of the National Institute of Food and Agriculture (NIFA) Agriculture and Food Research Initiative (AFRI) grants. In response to a USDA NIFA request for application to synthesize available results from these studies and from the literature, Cornell University was awarded a grant to convene a workshop in May 2010 to create an up-to-date picture of what we know about pathogens in rural and agricultural watersheds, and what remains unknown. The fundamental question that laid the foundation for the workshop breakout group discussions was, *What is the risk, in terms of pathogen load, to watersheds from farms and other sources?* Determining risk necessitated understanding the sources of pathogens and how they travel.

The Role of Agriculture?

The relationship among agriculture, drinking water, and public health has been researched extensively. Much scientific study has been undertaken in response to the need for safe drinking water (to protect public health) and clean watersheds (to prevent environmental degradation); the promulgation of federal regulations for animal feeding operations (AFOs); and public concern about water- and/or manure-associated outbreaks. While antibiotics, hormones, pesticides, and other farm-origin contaminants may affect human health, a significant focus of the research has been on pathogens originating in manure and their transport into groundwater and surface water.

The workshop examined multiple potential species and origins of pathogens in water supplies and attempted to assess their relative contributions. For the purpose of this paper, pathogens are defined as disease-producing organisms, and the pathogens with which we were concerned are those that cause disease in people. Pathogens from humans, livestock, wildlife, and pet waste are all potential sources for water contamination, as are resident pathogen populations already extant in the soil. Because this project was funded by the USDA, the primary goal was to determine the extent and impact of agricultural waste in watershed contamination. Specific attention was given to zoonotic pathogens of agricultural origin. Zoonotic disease transmission is a complex process that depends on transport, deposition, remobilization, and survival in multiple environmental reservoirs. Of equal importance are the biological properties of microbe and host that enable a microbe to be pathogenic in a particular species. The question of zoonotic risk is, therefore, complicated.

There is sufficient concern over livestock zoonoses and potential zoonoses to warrant investigation into how such pathogens may originate on and be transported from farms. Although most livestock manure is ultimately applied to land as fertilizer and soil conditioner, there is insufficient baseline information to establish up-to-date best management practices (BMPs) in this area; the time has therefore come to review and synthesize a substantial body of research. Guidance on how best to mitigate pathogen loads, be stewards of water resources, and protect consumer health should be provided to key stakeholders – both those owning facilities that may be sources of pathogens, and those consuming or otherwise utilizing water at risk of contamination.

For this discussion, it is critical to differentiate on-farm infiltration of manure into soils and water from slaughterhouse-based contamination of meat products. The processes and risks associated with each of these phenomena are quite different, and attempts to mitigate them are therefore also distinct. At slaughterhouses, animal feces attached to hides or leaking from perforated intestines can contaminate the meat being processed. That problem is beyond the scope of this watershed document. On farms, manure is often applied to the land as a natural fertilizer, and the pathogens contained therein may be able to leave the soil to which it is applied and enter water via runoff or other forms of transport. These are different phenomena and the risks to which they lead are quite distinct. Meat-associated outbreaks of zoonotic disease from packing plants are typically unrelated to how manure is managed on the farms, but rather to carcass management practices in the slaughterhouse or packing plant.

The Workshop

The intent of the workshop was to synthesize findings into a useful format for agricultural extension agents, consultants, policymakers, scientists, and farmers. An enormous amount of information exists in technical journals, conference proceedings, and grant reports, with limited accessibility to the people who can translate it into utilitarian actions. This document provides a useful summary of the most updated state of knowledge and noteworthy recommendations for stakeholders who can transform the information into meaningful actions and practical measures for compliance.

The workshop was held in Ithaca, New York, which is itself located in a rural watershed. The focus of the lectures and breakout groups was pathogens (bacteria, viruses, and protozoa) in rural and agricultural watersheds. Participants included NRI/AFRI-funded researchers, other specialists, and various stakeholders representing the farm industry. Their backgrounds included microbiology, environmental engineering, veterinary and human medicine, farming, and other expertises. Participants were divided into eight areas of expertise – 1) bacteria, 2) viruses, 3) protozoa, 4) transport, 5) disinfection, 6) watershed, 7) risk and regulation, and 8) producers. Each group met four times to work through four major topics – sampling methodologies, isolation and identification, transport and mitigation, and risk assessment and monitoring. By addressing specific questions about the state of the science in their breakout groups, participants provided answers that applied more generally to questions such as: What are we learning from basic research? What are we learning from applied research? What are existing BMPs and how well do they function? Each group then generated presentations that formed the basis of this synthesis document.

Goals

The intent of this report is to present our collective view of the state of the art in knowledge and research, best management practices, and recommendations for change. The project focused on meeting the goals of the USDA’s Water and Watersheds program: to protect and enhance the natural resource base and environment by improving and maintaining a healthy watershed, and to improve the quality of life in rural America through clean irrigation and livestock drinking water supplies. The project was designed to support water potability, good relationships between farmers and farm communities, cooperation between the government and the public and private sectors, and promote sustainable agriculture. Its near-term goal was to produce this summary of grant-funded and peer-reviewed information regarding pathogens in rural and agricultural watersheds.

A great deal of information has already been published on management practices, associated risks of pathogens from farms, and farm and water associated outbreaks; references for many of these studies are found in the Appendix. This report will not reiterate those valuable studies, and is designed to be complementary. It provides the knowledge and opinions of an assembly of experts, in order to bring the stakeholder community up-to-date on noteworthy scientific findings of the last several years, knowledge gaps, and recommendations for how to build on existing practices, regulations, and research initiatives. While some readers may be interested in the key findings presented at the end of each chapter and in the recommendations found in Chapter 7, most of the paper provides important background that was the basis for the workshop discussions and the recommendations that followed. We hope this report provides a valuable resource for decision-makers at the local, state, and federal levels for the next generation of guidelines and stakeholder discussions.

Less than 1% of the fresh water on the planet is available to us as surface water or groundwater. This report delineates risks and reduction parameters that will be useful for both regulators and educators to protect this water, and contains viable solutions for reducing and managing pathogen loading in the environment.

1

Chapter 1: Pathogens and the Watershed

All of the land on which we live, on which we grow our food, and that we enjoy for recreation resides in a watershed. Every city, every town, every park, and every farm calls a watershed home. This is true for all populations, whether in the central plains or on the coasts. Every area, whether as densely populated as the Mid-Atlantic or as sparsely inhabited as the Great Basin – all are contained within watersheds and all provide water for sustenance and for sport.

The United States Geologic Survey divides the nation into hydrologic units of four levels: regions, sub-regions, accounting units, and cataloguing units. The regions are depicted below (Figure 1).[5] The cataloguing units, also known as watersheds, amount to 2,264 distinct areas.

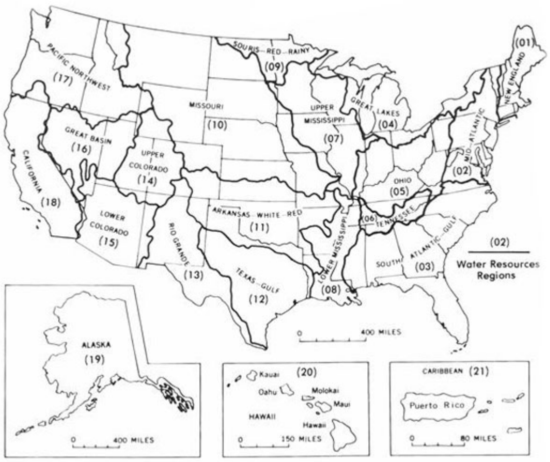


Figure 1: United States water resources regions

Source: United States Geological Survey Web site. http://waterwatch.usgs.gov/2009summary/images/us_map_large.jpg

---

[5] United States Geological Survey Web site. Water resources of the United States. Available at http://water.usgs.gov/GIS/huc.html. Accessed July 29, 2010.

These regions are not the only way to describe the nation's watersheds. An alternative approach developed by an intergovernmental panel (including USDA and USGS) and others, describes six "hydrologic units": 1) region, 2) subregion, 3) basin, 4) sub-basin, 5) watershed, and 6) subwatershed.[6] Together, these comprise the National Watershed Boundary Dataset.

It is not our intent to debate the merits of the different systems used to describe watersheds. We present these approaches simply to demonstrate that every parcel of land in the U.S. (and in every other country, for that matter) "lives" in a watershed. And despite the trillions of pathogenic organisms produced and excreted every day by people, livestock, and wildlife – and although a proportion of this excreta makes its way in one form or another into a watershed – the incidence of waterborne outbreaks of infectious disease is remarkably low.

Many barriers, both natural and manmade, are already in place to prevent outbreaks. These include areas of retention, dilution, and die-off between the pathogen source and the water supply. When manure is spread, it has typically been treated (via storage). Treatment of food and water prior to utilization and consumption presents a substantial barrier to pathogen transmission. Even when barriers are broken, the resulting contamination does not necessarily result in disease, or in the reporting of disease. Humans may be exposed but not clinically affected, and therefore the risk can go unnoticed. When outbreaks do occur, they may not necessarily be detected or reported. Finally, individual cases rather than outbreaks may occur, in which case the identification of a particular source is often not very likely.

Since 1971, the CDC, EPA, and Council on State and Territorial Epidemiologists have collaborated to maintain the Waterborne Disease and Outbreak Surveillance System.[7] Through this system, outbreaks are tracked to allow trends to be followed. Figures 2 and 3 and Table 1 display data for drinking water and recreational water outbreaks. A long-term analysis of drinking water outbreaks demonstrates that the number of outbreaks reported has come down considerably since a high in 1980. For the 2005-2006 season, the most recent for which an analysis is available, 78 waterborne disease outbreaks (recreational

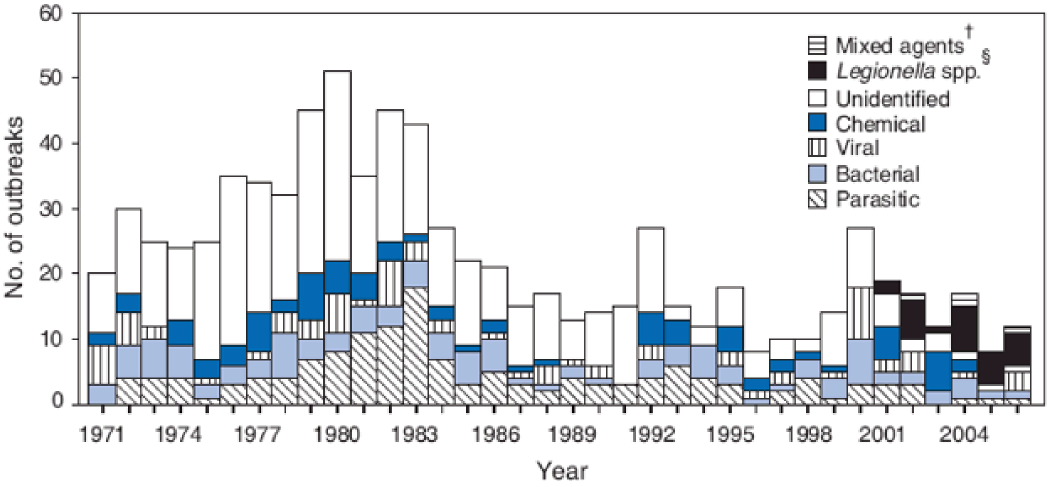
---

[6] Laitta MT, Legleiter KJ, Hanson KM. The national watershed boundary dataset. *Hydro line* Summer 2004:1.

[7] Yoder JS, Hlavsa MC, Craun GF, et al. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events — United States, 2005–2006. *MMWR* 2008;57/SS-9:1-70.

water) affecting 4,412 people were reported, the majority resulting in gastroenteritis. Parasitic, bacterial, and viral etiologies combined accounted for more than three-quarters of the cases in which a cause was identified. For drinking-water illness the same 2005-2006 season, twenty outbreaks caused illness in an estimated 612 people. An etiologic agent was identified in 18 of these drinking-water outbreaks – two thirds were bacterial, with the remaining being viral, parasitic, or mixed bacterial and viral. The CDC considers these outbreak numbers an underestimation (likely a result of underreporting), but lacks reliable estimates of the true burden of waterborne disease. (For perspective, compare these numbers to data for foodborne illness: for 2006, 1,270 outbreaks were reported, affecting 18,371 people.[8] The CDC estimates the actual yearly incidence of foodborne illness at 76 million people.[9])

Figure 2: Waterborne disease outbreaks associated with drinking water, by etiologic agent and year—United States, 1971-2006



* Single cases of disease related to drinking water (n = 16) have been removed from this figure; therefore, it is not comparable to figures in previous *Surveillance Summaries.*

† Beginning in 2003, mixed agents of more than one etiologic agent type were included in the surveillance system. However, the first observation is a previously unreported outbreak in 2002.

§ Beginning in 2001, Legionnaires' disease was added to the surveillance system, and *Legionella* species were classified separately in this figure.

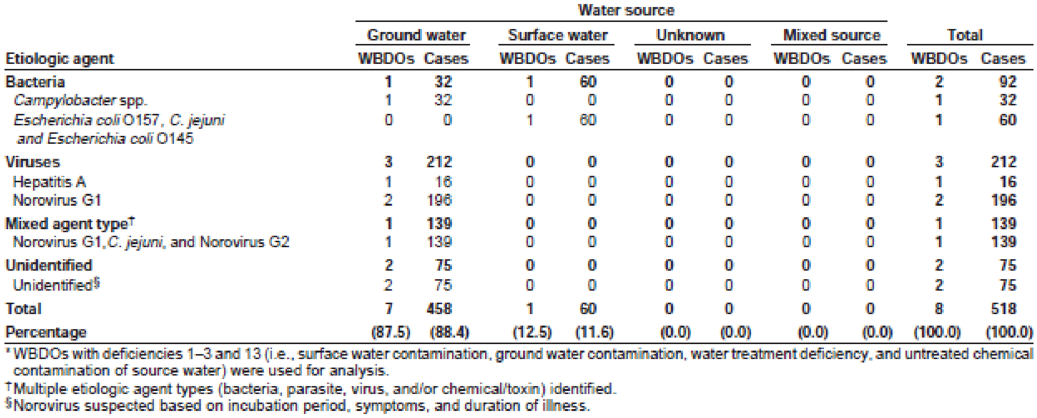
Source: Yoder JS, Hlavsa MC, Craun GF, et al. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events — United States, 2005–2006. *MMWR* 2008;57/SS-9:1-70.

---

[8] CDC. Surveillance for foodborne disease outbreaks—United States, 2006. *MMWR* 2009; 58:609-615.

[9] Centers for Disease Control and Prevention Web site. Foodborne illness. http://www.cdc.gov/ncidod/dbmd/diseaseinfo/foodborneinfections_g.htm. Accessed July 9, 2010.

Table 1: Waterborne disease outbreaks associated with drinking water, by etiologic agent and water source—United States, 2005-2006



	Water source									
	Ground water		Surface water		Unknown		Mixed source		Total	
Etiologic agent	WBDOs	Cases	WBDOs	Cases	WBDOs	Cases	WBDOs	Cases	WBDOs	Cases
Bacteria	1	32	1	60	0	0	0	0	2	92
Campylobacter spp.	1	32	0	0	0	0	0	0	1	32
Escherichia coli O157, *C. jejuni* and *Escherichia coli* O145	0	0	1	60	0	0	0	0	1	60
Viruses	3	212	0	0	0	0	0	0	3	212
Hepatitis A	1	16	0	0	0	0	0	0	1	16
Norovirus G1	2	196	0	0	0	0	0	0	2	196
Mixed agent type[†]	1	139	0	0	0	0	0	0	1	139
Norovirus G1, *C. jejuni*, and Norovirus G2	1	139	0	0	0	0	0	0	1	139
Unidentified	2	75	0	0	0	0	0	0	2	75
Unidentified[§]	2	75	0	0	0	0	0	0	2	75
Total	7	458	1	60	0	0	0	0	8	518
Percentage	(87.5)	(88.4)	(12.5)	(11.6)	(0.0)	(0.0)	(0.0)	(0.0)	(100.0)	(100.0)

[*] WBDOs with deficiencies 1–3 and 13 (i.e., surface water contamination, ground water contamination, water treatment deficiency, and untreated chemical contamination of source water) were used for analysis.

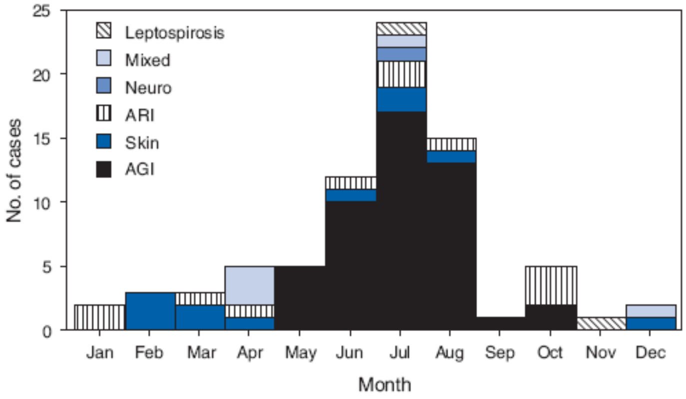
[†] Multiple etiologic agent types (bacteria, parasite, virus, and/or chemical/toxin) identified.

[§] Norovirus suspected based on incubation period, symptoms, and duration of illness.

Source: Yoder JS, Hlavsa MC, Craun GF, et al. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events — United States, 2005–2006. *MMWR* 2008;57/SS-9:1-70.

Recreational water-associated outbreaks tend to peak in summer months (Figure 3). In the 2005-2006 season, parasites accounted for the vast majority of identified causes, with *Cryptosporidium* spp. at the top of the list.

Figure 3: Recreational waterborne outbreaks by illness and month—United States, 2005-2006



* Mixed: a combination of illnesses, conditions or symptoms that also might include the eyes and ears; Neuro: neurologic condition or symptoms (e.g., meningitis); ARI: acute respiratory illness; Skin: illness, condition, or symptom related to skin; AGI: acute gastrointestinal illness.

Source: Yoder JS, Hlavsa MC, Craun GF, et al. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events — United States, 2005–2006. *MMWR* 2008;57/SS-9:1-70.

Pathogens were the culprit in many of these outbreaks. But what was the source of these pathogens? Simply demonstrating their presence in water does not offer up an answer to their origin. Unfortunately, origin is often not known or discovered for a particular outbreak. Furthermore, wastewater treatment plants in the U.S. are not required to monitor for pathogens in their effluent; they look only for coliforms. More epidemiological work is needed, with particular investment in non-point source testing of the many possible situations in which non-point sources are associated with real risk. Recreational water that may be impacted by runoff from agriculture, wildlife, domestic animals, or people may be ingested by bathers, surfers, etc. Research is currently defining the risks posed by these waters to those who come into regular contact with recreational water.[10,11,12] Although this and other work suggests that runoff-associated risks are few, there is little doubt that the risks are real in heavily used, agriculturally impaired waters. Thus, in these waters where there is reason for concern, it might be important to test for certain manure-associated pathogens to better quantify the magnitude of the risk.

1.1 Pathogens of Importance

Any discussion of the origin of pathogens in the watershed must begin with a designation of which of those pathogens are important to human and animal health. Although many pathogens may be found in water, the workshop discussions focused on those deemed most clinically relevant, those that have the clearest association with agriculture, and those about which we would like to know more. Despite this method of prioritization, it is nevertheless a challenge to rank these pathogens. Ranking imposes a somewhat artificial level of prioritization, which may then lead to artificial resource allocations. Just because a pathogen caused the most outbreaks does not mean that it caused the most severe disease or the highest fatality levels; just because a pathogen is commonly found in recreational water does not imply that other emerging pathogens will not soon overtake it. Also, for many of the pathogens, it is

---

[10] Stone DL, Harding AK, Hope BK, et al. Exposure assessment and risk of gastrointestinal illness among surfers. *J Toxicol Environ Health A* 2008;71:1603-1615.

[11] Sinigalliano CD, Fleisher JM, Gidley ML, et al. Traditional and molecular analyses for fecal indicator bacteria in non-point source subtropical recreational marine waters. *Water Res* 2010 Jul;44:3763-3772.

[12] Fleisher JM, Fleming LE, Solo-Gabriele HM, et al. The BEACHES Study: health effects and exposures from non-point source microbial contaminants in subtropical recreational marine waters. *Int J Epidemiol* 2010 Jun 3. [Epub ahead of print]

difficult to determine if the source is a farm, wildlife, or humans, and therefore where to target resources.

For this workshop, participants focused on manure-source pathogens commonly found in agricultural watersheds and deemed important to public health, animal health, or as tools for research. Table 2 represents those pathogens of known importance, given the above context. Table 3 lists those about which less is known but are worthy of monitoring or further study. Although helminths and fungi were considered as part of the initial proposal for this report, no helminths were ultimately included in either list, and only one fungus, Microsporidia, was considered important for its potential impacts, and is represented in the secondary list. Comprehensive lists (and discussions) of all pathogens found in watersheds can be found elsewhere. Smith et al.[13] provides an excellent resource on manure pathogens in the context of both agricultural and human health.

Table 2: Pathogens of importance

ORGANISM	TYPICAL SOURCE	NOTES
Viruses		
Enteroviruses	Human feces*	
Hepatitis A	Human feces*	
Hepatitis E	Swine feces	Sporadic in people in the U.S.; infections more common in developing countries
Myxovirus	Humans, Vertebrates	Mainly respiratory
Norovirus	Human feces*	Animal sources under investigation
Rotavirus	Human feces*	Animal sources under investigation
Bacteria		
Campylobacter jejuni	Poultry feces	
E. coli (enterohemorrhagic,	Cattle feces	

---

[13] Smith Jr. JE, Millner PD, Jakubowski W, et al., eds. *Contemporary perspectives on infectious disease agents in sewage sludge and manure*. The JG Press, Inc., 2005.

diarrheagenic, shiga-toxin producing); enteropathogenic (EPEC); enteroinvasive (EIEC); and enteroaggregative (EAgg EC)		
Salmonella enterica (serotypes Newport, Typhimurium, Enteritidis, Montevideo)	Cattle, poultry, and swine feces	
Protozoa		
Cryptosporidium		
C. hominis	Human feces*	No animal reservoir
C. parvum	Calf feces	Primarily calves less than 30 days old
Giardia duodenalis		
Assemblage A (I and II)	Human feces* and that of other mammals	AII only in people
Assemblage B	Human feces*	No animal reservoir

*Could enter rural and agricultural watershed through septic systems or municipal wastewater

Table 3: Pathogens of potential concern

ORGANISM	HOST(S)*	NOTES
Viruses		
Adenoviridae	People	Host specific; typically respiratory
Anelloviridae	People	
Astroviridae		
Mamastroviruses	Mammals	Host specific
Avastroviruses	Birds	Host specific
Birnaviridae		
Picobirnavirus	People, Pigs, Rabbits, Dogs, Cattle	
Caliciviridae		
Norovirus	Mammals	
Sapovirus	People	
Circoviridae		
Circovirus	Pigs	
Gyrovirus	Birds	

Coronaviridae	Mammals	Respiratory, SARS agent
Orthomyxoviiridae		
Influenza virus	Birds, Mammals	Respiratory, Flu
Picornaviridae		
Klassevirus	People	
Kobuvirus		
Aichi virus	People	
Bovine kobuvirus	Cattle	
Porcine kobuvirus	Pigs	
Enterovirus		
Coxsackievirus	People	
Echovirus	People	
Poliovirus	People	
Paraechovirus	People	
Cosavirus	People	
Reoviridae		
Rotavirus	People	
Bacteria		
Campylobacter coli	Poultry, Pigs, Cattle	
Campylobacter fetus	Cattle, Sheep, People	
Campylobacter lari	People, Sea gulls	
Campylobacter upsaliensis	Dogs, People	
Listeria monocytogenes	Cattle, Sheep, People	
Mycobacterium avium subspecies *paratuberculosis*	Cattle, Ruminants	
Shigella	People	
Vibrio cholera non-01	Sea and coastal waters	Consuming seafood
Yersinia enterocolitica	Cattle, Deer, Pigs	
Protozoa		
Balantidium coli	People, Pigs	
Blastocystis sp.	People, Pigs	
Cryptosporidium		
C. andersoni	Cattle	Rare in people
C. bovis	Cattle	Rare in people
C. meleagridis	Turkeys, birds	Rare in people
C. ryanae	Cattle	Rare in people
C. ubiquitum	Mammals, including	Rare in people

	People	
C. xiaoi	Sheep, Goats	Rare in people
Cryptosporidium horse genotype	Horse	Rare in people
Giardia duodenalis		
Assemblage C	Dogs	
Assemblage D	Dogs	
Assemblage E	Hoofed stock	
Assemblage F	Cats	
Assemblage G	Rats	
Toxoplasma gondii	Cats	
Fungi		
Microsporidia	Mammals	

*Host in which most commonly found

These pathogens have been listed in order to provide readers with a context for the following chapters. Although this document does not intend to comprehensively discuss the *microbiology* of all pathogens found in watersheds, it is important to emphasize a few points about high priority pathogens and pathogens for which knowledge gaps still exits and more research would be useful.

1.1.1 Bacteria

In the past decade, multidrug-resistant *Salmonella enterica* serovar Newport has undergone a rapid epidemic spread in animals and humans in the U.S. This particular *Salmonella* serotype has apparently established a reservoir in dairy cattle, and although the true herd prevalence of this organism is unknown, it has been identified in all states of the continental U.S. Infected animals shed the bacteria in manure continuously or intermittently for weeks or even months. One recent study has shown post-shed survival of the organism in the laboratory as well as in natural environments for weeks, and potentially for more than a year.[14] Transport of the organism through leaching readily occurs under simulated rainfalls. The fact that *S. enterica* Newport is resistant to multiple antibiotics, has established a reservoir in dairy cattle, can survive long periods

---

[14] You Y, Rankin S, Aceto H, et al. Survival of *Salmonella enterica* Serovar Newport in manure and manured-amended soils. *Appl Environ Microbiol* 2006;72:5777-5783.

post-shedding, and moves readily with water from manured soils makes the organism of particular concern to the public health community.

Pathogenic forms of *E. coli* are another significant public health concern. A major thrust of the USDA in the next few years will be the management of shiga toxin-producing *E. coli* (STEC). One set of projects will focus on the microbial ecology and STEC shedding in cattle. This research will focus on evaluating changes in microbial communities within the rumen in association with shedding of STEC by cattle (at multiple operations, locations, and management practices), and other studies will focus on the effects of season, stress, management, and feed type on STEC shedding. A larger set of competitive projects is focused directly at protecting the consumer. These very large projects (funded at a cost of up to $25 million for five years) will identify risk factors for STEC contamination in the pre-harvest and post-harvest environments, develop intervention and risk management strategies, and evaluate the effectiveness of the strategies. A particular goal is the validation and/or development of detection methods that determine viability and that are rapid, sensitive, specific, economical, portable, and easily conducted, and demonstrated to work in food and/or environmental samples. In this way, the USDA through its AFRI research funding will directly address this concern of the American consumer.[15]

1.1.2 Viruses

Although noroviruses are one of the most common viruses from a clinical standpoint, they do not typically originate in agricultural settings. Current evidence suggests that noroviruses that infect people come only from other people, transmitted by the fecal-oral route through contaminated food or water. Noroviruses typically cause food-borne outbreaks ("food poisoning") but they are also occasionally the cause of waterborne outbreaks. The zoonotic capabilities of noroviruses are not completely understood, although the virus has been isolated from calf and swine feces. We are only just beginning to understand the full diversity of noroviruses in animals, and their ability to transmit to people either directly or through recombination events.

Gap: We lack detailed knowledge of the zoonotic capabilities of noroviruses. [This gap was already identified and is currently a focus of the USDA's ongoing research effort. Under the NIFA AFRI initiative in Food Safety, grants are being provided for *Prevention, Detection, and Control of Food-borne Viruses in Food: A*

---

[15] For information on these grants, see: http://www.nifa.usda.gov/funding/rfas/pdfs/10_afri_foodsafety.pdf

Focus on Noroviruses. Proposals are being sought to develop studies to evaluate the occurrence and fate of noroviruses and other food-borne viruses in food to assess their potential risk to food safety and human health. We encourage studies funded under these grants to address the zoonotic potential of noroviruses as related to livestock and wildlife.]

Increasing evidence supports the hypothesis that hepatitis E virus (HEV) is a zoonotic infection.[16,17,18] Infection with HEV is of particular concern in pregnant women, in whom the case fatality rate can be quite high. Most outbreaks have occurred under highly unsanitary conditions in the developing world and are associated with feces-contaminated drinking water. U.S. cases usually have history of travel to HEV-endemic areas, primarily in developing countries with inadequate environmental sanitation. HEV is endemic in regions where the water supply may be contaminated with animal waste, such as in Central Asia, the Middle East, and parts of South America and Africa. The reservoir of HEV in these areas is unknown. The occurrence of sporadic HEV infections in humans may maintain transmission during inter-epidemic periods, but a nonhuman reservoir for HEV is also possible. The virus has been isolated from pigs in many developed countries. In addition to swine, HEV is found in sheep, and an HEV-like virus has been isolated from chickens and rodents.[19,20]

Gap: Despite significant investment by human health agencies, we lack broad knowledge of the biology, epidemiology, and serology of HEV, in animals other than swine.

1.1.3 Protozoa

Animal and farm sources were initially implicated in many outbreaks of cryptosporidiosis and giardiasis. A significant amount of subsequent research, led to a great extent by the USDA Agricultural Research Service laboratories and the CDC, has shown that the majority of infections with these pathogens, at least

---

[16] Brost S, Wenzel JJ, Ganten TM, et al. Sporadic cases of acute autochthonous hepatitis E virus infection in Southwest Germany. *J Clin Virol* 2010;47:89-92.

[17] Meng XJ. Hepatitis E virus: animal reservoirs and zoonotic risk. *Vet Microbiol* 2010;140:256-65.

[18] Meng XJ, Wiseman B, Elvinger F, et al. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. *J Clin Microbiol* 2002;40:117-122.

[19] Vitral CL, Pinto MA, Lewis-Ximenez LL, et al. Serological evidence of hepatitis E virus infection in different animal species from the Southeast of Brazil. *Mem Inst Oswaldo Cruz* 2005;100:117-22.

[20] Johne R, Plenge-Bönig A, Hess M, et al. Detection of a novel hepatitis E-like virus in faeces of wild rats using a nested broad-spectrum RT-PCR. *J General Virol* 2010;91:750-758.

in the U.S., are due to human-to-human transmission. There is a farm-associated species of *Cryptosporidium*, *C. parvum*, that poses a significant risk to people, but most infections occur in people placed at risk through direct contact with infected calves (e.g., petting zoo-goers, veterinary students, dairy workers). Similarly, the assemblages of *Giardia* found in hoofed-stock are not those that typically infect immuno-competent people. Occasionally, cattle can shed human-type *Giardia* (i.e., Assemblages A or B), but this is rare, and the *Giardia* in the cattle in these instances was likely acquired from their human handlers. *Balantidium coli* of pigs has not been studied in any great detail. Although pigs and people can both be infected, there have been no water-associated outbreaks described and infections in people in the U.S. are rare.

1.2 Sources

Pathogens that enter a watershed originate with animals (including people) and are then transported within and through the watershed. (Some refer to host organisms as "primary" sources and environmental reservoirs as "secondary" sources.) Animal sources of pathogens that enter the watershed include:

- <u>humans</u> – leakage/effluent from septic tanks, sewers, wastewater treatment plants;[21] recreational water use
- <u>domesticated animals</u> – runoff from land-applied livestock manure, direct deposition of manure or feces into on-farm or adjacent streams
- <u>wildlife</u> – runoff from feces deposited in agricultural fields, feces deposited directly in streams, or on beaches; waterfowl contamination of open water bodies

A variety of storage media and transport processes can then carry pathogens to the point of exposure. These include:

- sewage sludge (biosolids)[22] (e.g., application to farm lands)
- dust
- irrigation water
- soil
- water bodies (e.g., groundwater, ponds, and rivers)

---

[21] Wastewater treatment plants are not required to monitor for pathogens in their effluent.

[22] In this report, biosolids are defined as sewage sludge, typically after subsequent processing to facilitate re-use.

- stream bed sediments and biofilms
- tile drains
- vectors (such as insects)
- wash water

No single source is likely to be uniquely responsible for pathogenic contamination of a water body. The prevalent sources vary by type of watershed (e.g., upland versus coastal), type of land use (e.g., agricultural versus urban), agricultural practices, topography, soil characteristics, etc. Nevertheless, runoff is considered a primary mechanism of pathogen delivery, and frequently occurs in many AFOs and confined animal feeding operation (CAFO) systems. Even "zero discharge" designs may have discharges during relatively high precipitation events.[23] Large flow events are a particular problem, as systems are not normally designed to be resistant to floods; farms have limited storage capacity, and are therefore vulnerable to overflow during floods. Some studies have examined relative loads from different sources, but in general it is not known how magnitudes of releases are distributed in small/distributed versus large/concentrated animal operations. Thus, more research, in particular for monitoring, may be warranted in this area.

In addition to being considered as primary or secondary sources of watershed input, sources may also be described as either point sources or non-point sources. Point sources are most easily summarized as a single defined source, such as the outflow from a single pipe or drain; non-point sources are from movement across terrain, a spray-field, irrigation water, or contamination that leaves an area via surface water runoff, groundwater contamination, etc. General releases from agricultural land surfaces appear to be a large non-point type of source.

The EPA assesses more than 30 causes of water quality impairment of water bodies in the U.S.[24] Water bodies include rivers, streams, lakes, estuaries, coastal waters, etc. Causes of impairment listed include pathogens, sediment, nutrients, habitat alterations, pesticides, mercury, etc. The primary cause of impairment varies by water body. For example, based on miles of rivers and streams threatened or impaired, pathogens rank as the most probable source of

---

[23] Even CAFOs that have permits and zero discharge plans are allowed to land apply manure to crop fields. The zero discharge permit applies only to the CAFO structure and manure storage area, not the manured fields.

[24] United States Environmental Protection Agency Web site. Watershed Assessment, Tracking and Environmental Results. Available at http://iaspub.epa.gov/waters10/attains_nation_cy.control. Accessed June 24, 2010.

impairment, affecting 141,702 miles. (Fecal coliforms are listed as responsible for more than half of those miles, followed by *E. coli* and then a generic grouping called "pathogens." Of the 22 probable sources of these pathogens, "agriculture" tops the list. "Unknown" cause is second. "Natural/wildlife" is fifth. Human-based sources contribute in a variety of ways and rank at a variety of levels.) Contrary to this, for lakes, reservoirs, and ponds, mercury is listed as the major culprit of impairment, resulting from atmospheric deposition and a variety of other causes, many of which are unknown or poorly quantified. The causes and sources vary for the other categories of water bodies.

The microbes for which the EPA sets regulations, and for which state agencies and municipal water suppliers monitor, are primarily *E. coli and* fecal coliforms. In some cases, the presence of *Enterococcus* and *Cryptosporidium* is also monitored. When the source is human excrement, fecal coliform levels have been shown to be indicators of co-contamination with human pathogens (i.e., microbes that actually result in disease). The association, however, between the presence of *farm*-derived coliforms and farm pathogens is less well established. Furthermore, the likelihood that human bacterial pathogens are present in animal manure is less than that in human sewage. While *E. coli* is indeed found in animal manure, aside from the few strains such as *E. coli* O157:H7 that causes serious disease in people, most *E. coli* are not pathogenic. Furthermore, the data presented by the EPA include *E. coli* from wildlife, livestock, and people; assessing agriculture in particular as a primary source is difficult without subtyping or other source tracking technology. It is well known that migratory birds, endemic rodent and deer populations, raccoons, opossums, and other wildlife contribute large quantities of fecal matter to watersheds. This makes it very difficult to determine to what extent farms are the specific source of the impairment, and they should not, therefore, be automatically cited as a predominant source of the impairments with specific reference to pathogens.

It was frequently noted during the workshop that more monitoring is needed. The question is, monitoring for what? And where – how do we decide the geographic locales and water bodies that warrant monitoring? And who will do it? Much has been learned about which pathogens are transported off the farm to certain water bodies, and how this happens, but widespread application of this information is limited by current capability for environmental measurement of pathogens. Advanced monitoring technology that quantifies specific pathogens rather than indicator bacteria is available, but is costly and pathogen-specific. Monitoring is often designed to measure pathogen presence at water supply plants and intakes, but these strategies do not identify the source animal or location within the watershed. This becomes especially problematic in large watersheds where sources become mixed. So, while many participants were enthusiastic about monitoring (see Chapter 4), practical limitations may pre-empt efforts in this area.

A simple test that could sample water for pathogens of interest and determine the pathogen and its source – human, cattle, swine, chicken, or wildlife – would be extremely beneficial. However, there is still the matter of who would do the monitoring. Monitoring is done mainly through the EPA's mandate under the Clean Water Act, with most being done by point source dischargers such as sewage treatment plants with effluent streams entering waterways. Except for specific cases where intensive monitoring is conducted to support watershed planning, remediation, or other special projects, watersheds are typically monitored by regional water management districts, volunteer organizations working with the EPA, NSF, or the extension services of the USDA. Alternatively, the Safe Drinking Water Act requires source water testing by drinking water systems. Rigorous monitoring would present major costs that would have to be borne by the farmer, by the consumer in increased costs of farm goods, or the taxpayer. Even at the research level, costs are sometimes too high to allow for basic data collection.

Gap: Scientists interested in performing monitoring for research frequently lack the budget for state-of-the-art equipment or for manpower for regular sampling. It would be helpful for USDA grants to allow sufficient funding for such purposes.

1.2.1 Animal sources

FECES: Excrement that is produced by the body and which leaves the body through the anus. Used as both a singular and a plural noun. The Latin singular is *faex* which represents a single fecal product, such as one rabbit, sheep, or deer pellet.

MANURE: A mixture of bedding, animal dung, urine, and feed refusal plus other materials such as soils and waste water. Derived from the Latin, meaning to work by hand or till the soil. Manure has almost always received some form of processing before it is land applied. It has historically been stacked, dried, lagooned, composted, frozen, digested, heated, or sometimes burned prior to land application.

Animal wastes arise from domestic livestock, pets, and wildlife. Attention to farms as a particular source of pathogens from waste has increased in recent years. Operations have become fewer in number but larger in size, and more industrialized in scope. Collectively, the nation's livestock farms produce enormous amounts of animal waste. According to the USDA, the amount of manure generated at AFOs and CAFOs is estimated to exceed 335 million tons of dry matter per year.[25]

To put this in context, simple calculations allow a comparison between the waste produced by livestock to that produced by dogs and cats. In 2007, there were more than 72 million pet dogs in the U.S. and nearly 82 million pet cats.[26] If each of the 72 million dogs produce an average of 0.2 kg feces/day, that amounts to 5.3 million metric tons/year. For 82 million cats each producing 0.05 kg feces/day, that yields 1.5 million metric tons/year. For the 307 million people in the U.S., with an average output of 0.5 kg feces/day, the product is just over 56 million metric tons per year. The output numbers for livestock given by the USDA are based on a dry matter calculation. On the basis of the assumption that the fecal material of dogs, cats, and people is about one-third dry matter, the millions of metric tons of dry matter produced by dogs, cats, and people is 1.8, 0.5, and 18.7 million metric tons/year, respectively. While livestock therefore produce much more waste, that produced by people and their pets is not insignificant, and costs associated with treating human waste can be quite high. In the 1970s and 1980s, the federal government assisted municipalities with billions of dollars to improve infrastructure for sewage treatment to help offset these costs.

The public perception of large-scale, waste-producing farm operations leads to something of a contradiction. Clean drinking and recreational waters are highly demanded, but consumer pressure for organic (in this context, non-synthetic fertilizer-based) farming is also increasing. Also, the mandate that cattle be allowed access to grazing land leads to the increased presence of cattle in associated brooks and pastures from which runoff can occur. The scale of large farms as opposed to small holder farms also makes manipulation of large volumes of manure cost effective.

Public perception is critical, as it is often a driver for policy-making. A front-page *New York Times* article on *E. coli*, published the week after the workshop took place, stated:

---

[25] United States Department of Agriculture Web site. National Program 206: manure and byproduct utilization. Available at http://www.ars.usda.gov/research/programs/programs.htm?np_code=206&docid=13337. Accessed July 26, 2010.

[26] American Veterinary Medical Association Web site. U.S. pet ownership and demographics source book (2007 edition). Available at http://www.avma.org/reference/marketstats/sourcebook.asp. Accessed July 26, 2010.

> *"It is not clear how E. coli travels from cattle to produce, but scientists think it may occur through contact with manure, perhaps tracked through fields by wild animals, or through tainted irrigation water."*[27]

Although it has been demonstrated that pathogens from an animal operation can be transported to adjacent watersheds, and it is certainly believed anecdotally, the extent to which farms are actually the source of human disease outbreaks continues to be debated and studied. The EPA has attributed much of the pathogenic contamination of water bodies to manure production at CAFOs.[28] The EPA, however, primarily regulated fecal coliforms and *E. coli*. "Pathogens" and "*E. coli*" are not necessarily synonymous. And we cannot ignore septic tanks in favor of indictment of farms. In 2007, 20% of households in the U.S. were served by soil-based septic systems, half of which were in rural areas.[29] Up to 10% of the septic systems in the U.S. have stopped working, creating a prominent source of ground water contamination.[30] When they fail, nutrients and pathogens are the primary pollutants released from such systems. A comparative study of residential wastewater treatment in old versus new septic tanks, and AFO waste streams within the same watershed, would be beneficial to elucidate the extent to which farms are the source of human disease outbreaks.

Manure is a key focus of study because it is replete with microbes, some of which are human pathogens that can enter the environment through land manure applications, manure lagoons, or manure storage, to name a few pathways. Manure management, other farm-level and watershed-scale management practices, and geomorphology all affect transport. The role wildlife and their waste play in outbreaks is not elucidated; it is likely important but even less well understood than the role of livestock. The potential interactions and relative contributions of the two groups and their pathogens are also minimally understood, but some study has been undertaken.

---

[27] Neuman W. In E. coli fight, some strains are largely ignored. *NYT* 26 May 2010:A1.

[28] United States Environmental Protection Agency. R*isk assessment evaluation for concentrated animal feeding operations*. Cincinnati: National Risk Management Research Laboratory, 2004.

[29] United States Environmental Protection Agency. Office of Wastewater Management. *Septic systems fact sheet*. EPA# 832-F-08-057 Oct. 2008. Available at http://www.epa.gov/owm/septic/pubs/septic_systems_factsheet.pdf

[30] United States Environmental Protection Agency. *Septic (onsite) systems: frequent questions.* Accessed August 13, 2010. Available at: http://cfpub.epa.gov/owm/septic/septic.cfm?page_id=261#368

As a case in point, McEvoy[31] investigated sources of *Cryptosporidium* in a river in North Dakota. *C. andersoni* (not zoonotic) was the only species identified in water downstream of a cattle ranch, but was rarely found upstream. Conversely, wildlife genotypes predominated upstream but were not identified downstream. It is unlikely that wildlife genotypes are absent downstream, but rather that *C. andersoni* is present at high levels and selectively amplified during PCR. The researchers developed an HSP70 subgenotyping method, which may prove useful for further studies of pathogen contamination.

This study is one of the first to evaluate wildlife genotypes and water in the same area. The *Cryptosporidium* muskrat genotype I and vole genotype were present in small mammals and adjacent river water samples, and both genotypes were also found in meadow voles. The relatively large abundance of muskrats and voles and the fact that their genotypes were isolated in the water samples indicate their role as a source of water contamination. Although the muskrat and vole genotypes are not known to infect humans, *C. parvum* and the cervine genotype were also identified in river water samples and these species have been positively associated with human cryptosporidiosis.

In the context of what were predominantly agricultural conversations at the workshop, this study provides excellent reminders that more wildlife research is needed, and that assessments of genus and genotype are critical in epidemiological study to link human disease to a particular source.

Gap: More research is needed on wildlife-transmitted pathogens and the proportion of waterborne pathogens attributable to wildlife.

One pathogen for which it is certain that wildlife (and companion animals) play a role is *T. gondii*. Farm animals are not a source of environmental stages of *T. gondii*. The only source of the oocysts of *T. gondii* is the fecal material of cats and other wild felids. There have been a few reported water-borne outbreaks, the most notable having occurred in Vancouver, BC, Canada.[32] Oocysts are also making their way into wildlife, such as sea otters, where they cause significant disease.[33]

---

[31] McEvoy JM. Sources of *Cryptosporidium* in a rural North Dakotan river. Contract no.: 2006-35102-17237.

[32] Bowie WR, King AS, Werker DH et al. Outbreak of toxoplasmosis associated with municipal drinking water. *Lancet* 1997;350:173-177.

[33] Miller MA, Miller WA, Conrad PA, et al. Type X *Toxoplasma gondii* in a wild mussel and terrestrial carnivores from coastal California: new linkages between terrestrial mammals, runoff and toxoplasmosis of sea otters. *Int J Parasitol* 2008;38:1319-28.

Balantidium coli is found in people, pigs, and a few types of rodents. Pigs are considered a reservoir host of infection by many, but it is unknown to what extent cross-transmission occurs, or whether the species *B. coli* is actually a number of distinct species (as was found to be the case with the molecular dissection of *C. parvum*). It may be that in the developed world, most infections are anthroponotic (carried among humans with the potential for transfer to other animals) without the need for swine reservoir hosts. This scenario seems likely because pig manure is handled in such a way that the cyst stage of *B. coli* would normally be inactivated.[34]

Blastocystis sp. are protozoa that occur in the colon of people and some other animals, including pigs. (Current usage often omits the name *Blastocystis hominis* in the literature in favor of *Blastocystis* sp.; there are so many genotypes within this "umbrella" that are likely independent species, making the designation of *B. hominis* misleading. In this paper the name is used only if a particular study naming *B. hominis* is being cited.) There is some concern that these may be water-borne pathogens, but at this time, they have not been linked to any animal sources as strict reservoirs of infection.[35] (One recent small study demonstrated people, their pets, and their tap water to all have the same subtype of *B. hominis*.[36]) Thus, it appears that there may be cross-transmission of this agent between species, but this work is in early stages.[37] It also appears that *Blastocystis* sp. is one of the few members of the group of organisms known as Stramenopiles that are internal parasites of vertebrates.[38]

Microsporidia are fungi that can infect mammals and birds, and are transmitted between hosts as spores shed in feces, urine, and other body secretions. It is unclear to what extent, if any, domestic farm animals serve as sources of human infection, although several genotypes of *Enterocytozoon bieneusi* appear genetically identical in some animals and humans, based on a

---

[34] Schuster FL, Ramirez-Avila L. Current world status of *Balantidium coli*. *Clin Microbiol Rev* 2008;21:626-638.

[35] Suresh K, Tan TC, Illi, F. Blastocystis in water - need for screening? *Water Prac Tech* 2009;4.

[36] Eroglu F, Koltas IS. Evaluation of the transmission mode of *B. hominis* by using PCR method. *Parasitol Res* 2010 Jun 11. [Epub ahead of print.]

[37] Parkar U, Traub RJ, Vitali S, et al. Molecular characterization of *Blastocystis* isolates from zoo animals and their animal-keepers. *Vet Parasitol* 2010;169:8-17.

[38] Riisberg I, Orr RJ, Kluge R, et al. Seven gene phylogeny of heterokonts. *Protist* 2009;160:191-204.

single gene sequence. Work is progressing in this area, and some spores have been recovered from drinking water sources in the developing world.[39]

Gap: There is insufficient epidemiological data on the importance of waterborne transmission of *T. gondii, B. coli, Blastocystis* sp., and microsporidia*,* and the sources of the latter three pathogens are not well understood.

Understanding the diversity of animal and human pathogen strains, isolates, assemblages, genotypes, etc. and where they merge and diverge will help elucidate the risk posed by different domestic animals as potential sources of watershed contamination. To a great extent due to USDA-funded research, it has become clear that the risk of transmission of *Giardia* and *Cryptosporidium* from farm animals to people is not as great as was suspected only ten years ago. For instance, the work has shown that it is almost exclusively young, preweaned calves that shed the species of *Cryptosporidium* (*C. parvum*) that can readily infect people. There is great interest in the possibility that additional organisms may be shared by people and pigs, cattle, and poultry, but this research showing the potential of shared viruses, bacteria, microsporidia, and organisms such as *Blastocystis* sp. is just emerging. The application of modern molecular methods to these systems may help elucidate answers to the question of zoonotic risk.

1.2.2 Human sources

Humans account for a significant proportion of waste as well – just over 300 million people in the U.S. produce about 60 million metric tons of feces per year. Human sewage has been implicated in infectious disease outbreaks, via mechanisms such as breached septic and sewer systems, residential waste stabilization lagoons,[40] and contamination through use of recreational waters. Illegal dumping may also be a source of watershed contamination. There is every reason to believe that human sewage is a more common source of infection of other humans simply due to the adaptation of human pathogens to human hosts.

---

[39] Dowd SE, John D, Eliopolus J, et al. Confirmed detection of *Cyclospora cayetanesis*, *Encephalitozoon intestinalis* and *Cryptosporidium parvum* in water used for drinking. *J Water Health* 2003;1:117-123.

[40] Schultheis RA. Residential Sewage Lagoon Systems: A Homeowner's Guide to Installation and Maintenance. Available at http://extension.missouri.edu/publications/DisplayPub.aspx?P=WQ402. Accessed July 6, 2010. The paper describes how such lagoons are commonly used for on-site sewage treatment in Missouri in low population areas where soils are ill-suited for traditional systems; malfunctioning of the lagoons is believed to be pervasive. These lagoons can be found in other states as well.

The source of human infection varies by country, not only because of geographic variations, but also in relation to economic development. For example, the transmission of *C. parvum* in humans is mostly anthroponotic in developing countries. Zoonotic infections, however, play an important role in developed countries, although the majority of cases are still anthroponotic except in farm-associated outbreaks. Most human cases of anthroponotic and zoonotic cryptosporidiosis worldwide are associated with the two species, *C. hominis* and *C. parvum*.

Poliovirus, a pathogen of humans and some other primates, has been reduced as a disease of humans through two modes of prevention: inactivation by sewage treatment, and vaccination. Unfortunately, as we have reached the point where the disease is rare, the ease of sewage transmission between people has been made evident by the rapid spread of vaccine-induced poliovirus in unprotected populations.[41] With human-adapted pathogens, spread can be remarkably efficient due to the ability of people to serve as sources of very large numbers of infective units that can infect other individuals.

When one looks at the tables below that show the pathogens of interest and pathogens of concern that are generated in rural and agricultural watersheds, it is clear that a number of the agents are *human* viruses, bacteria, and protozoa. A number of these are relatively new to the world of science and agriculture. Molecular methodologies have allowed a mammoth upsurge in identification of organisms in the last 20 years, and have also allowed the determination that some organisms seem to be identical when isolated from people and animals. Thus, we are looking at the ability of farm animals to serve as sources of recombination of viruses with human strains, sources of antibiotic resistant agents that might infect people, and agents such as microsporidia that were once not considered of human pathogenic importance except in very rare instances. It needs to be remembered that people are a major part of watershed, not just animals.

In New York, it was realized that municipalities are potential sources of pathogen contamination of watersheds, and water treatment facilities there have been assisted in placing microfiltration systems for water discharged from wastewater treatment plants.[42] However, the nation's numerous septic systems

---

[41] CDC. Update on vaccine-derived polioviruses--worldwide, January 2008-June 2009. *MMWR* 2009;58:1002-1006.

[42] Habib J, Reightmyer R. Upgrading wastewater facilities with membrane microfilters. Waterworld Web site. Available at http://www.waterworld.com/index/display/article-

are not likely to undergo significant upgrades around a watershed. Also, many communities are having trouble maintaining current wastewater infrastructure, let alone adding the costs of additional tertiary treatment processes to their municipal budgets. Thus, it is unlikely that there will be major reductions in the human pathogens entering these watershed systems. Human fecal matter (along with the large volumes of feces from companion animals and wildlife) will continue to enter the nation's watersheds.

1.3 Relevance of Pathogens in the Watershed

1.3.1 Animal health

It was not so long ago that many people lived in the same structure as their working animals, or in otherwise very close contact (Figure 4). Fewer than 100 years ago, many people in America were routinely in contact with horse, cattle, and pig feces. Until the invention of the automobile, even large U.S. cities routinely employed horses and oxen for moving goods and people, and city blocks were devoted in many cities to holding animal waste in giant piles until it could be hauled away. At times, New York City may have contained as many as 100,000 to 200,000 horses.[43] Thus, in the relatively recent past, the chance for the transfer of pathogens from domestic animals to people via contact with animal feces was markedly greater than today. An

Figure 4: Illustration of an outhouse on an early 20th century farm

Source: Wardell Stiles C. *Soil pollution as cause of ground-itch, hookworm disease (ground-itch anemia), and dirt eating.* Washington, DC: The Rockefeller Sanitary Commission for the Eradication of Hookworm Disease, 1910.

---

display/322065/articles/membranes/volume-1/issue-1/feature/upgrading-wastewater-facilities-with-membrane-microfilters.html. Accessed August 23, 2010.

[43] The Living City Web site. Interview with David Rosner – Portrait of an unhealthy city: New York in the 1880s. Available at http://www.tlcarchive.org/htm/framesets/living_city/fs_dev.htm. Accessed July 27, 2010.

implication of this change, however, may be that reduced fecal exposure reduces our ability to protect ourselves against infections with fecal pathogens due to the lack of priming of our immune systems by constant exposure.[44,45]

A variety of on-farm practices may be important for controlling the spread and recycling of animal infection, including source of water, water piping systems, pasture management, bedding recycling, use of animal products in feed, feed storage practices, sanitation of transportation vehicles, and management of contact between current and new animals. The potential for disease transmission increases with intense/high-density sources, and also with potential for rapid transmission of high-concentration pulses of pathogens (e.g., flood-prone areas, karst aquifers, etc.).

Farmers have worked at reducing the disease present on farms that interfere with their own production and the health of their animals. For this reason, we live in a country free of foot and mouth disease virus. We have eradicated from the U.S., through the research and control efforts of the USDA, Texas tick fever, hog cholera, and the deadly form of viscerotropic Newcastle disease virus of poultry from the U.S. Through vaccination and testing, many diseases such as brucellosis and trichinellosis are essentially diseases of the past. In large part due to improved diagnostic methods, however, it has been realized that a number of agents that have little or no impact on the health of farm animals do, on occasion, cause disease in people. The agents that have caught the greatest attention have been *Cryptosporidium*, *Giardia*, *Salmonella*, and *E. coli*. The public is aware that these and other agents threaten their water and food supplies and can cause significant disease. It has become important to determine means of minimizing the risk of the presence of farming on the surrounding public.

Rural, suburban, and urban boundaries have blurred in recent years, as farms and urban water supplies encroach upon one another, and as housing developments infiltrate farm communities. This phenomenon has brought together, and sometimes into conflict, people who have different objectives and goals for the community's land and water use. Farmers have long been wrestling with this issue, most recently through CAFO regulations that required permits for nutrient management. A great deal of effort has recently gone into reducing the potential of pathogens leaving the farms through improved buffer zones,

---

[44] Okada H, Kuhn C, Feillet H, et al. The ‘hygiene hypothesis’ for autoimmune and allergic diseases: an update. *Clin Exp Immunol* 2010;160:1-9.

[45] Björkstén B. The hygiene hypothesis: do we still believe in it? *Nestle Nutr Workshop Ser Pediatr Program* 2009;64:11-8; discussion 18-22, 251-7.

manure handling, and manure storage. Farmers are very interested in using manure as a potential source of energy production. Also, work continues on vaccines that would minimize some of the pathogens in calves and piglets, such as *Cryptosporidium*, noroviruses, and rotaviruses, that would minimize the shedding of these agents from a farm community. The specific targeting of these animals may markedly minimize the organisms produced on any given farm.

Watershed protection is not an easy task for the farmer. Although a contingent of the public is against large farms, the tradeoff is that it is difficult to control the effluent from a large number of small farms. Neonatal infections in cattle, sheep, goats, and pigs, are very similar to neonatal infections in people, in that they occur routinely and commonly. Thus, a large number of calves will shed a large number of organisms, whether there are 30 calves on 100 farms or 300 calves on 10 farms. It must always be remembered that farms are for-profit operations, and unlike municipal water and wastewater treatment plants, there are not taxpayer dollars being used on a regular basis for risk management from individual farms.

Farmers have been working hard to minimize the risk to their neighbors and their communities, and these efforts are likely to improve and increase due to the desire to be good stewards or their land, to the pressure applied by surrounding communities and watershed organizations, and to the fear of being responsible for making people ill. It is highly likely that farmers will continue to implement processes that minimize runoff, that reduce aerosols, and that reduce pathogen loads, and enhance pathogen inactivation. The USDA will continue to mold farm production in a way that will protect the U.S. land, water, and people.

On-farm recycling of pathogens, however, is an essentially unavoidable part of doing business. Completely eliminating exposure is difficult, and once a pathogen is contracted, fecal shedding then exposes other animals. Minimizing pathogen reservoirs on farms and rangeland, and targeting routes of exposure, are therefore key intervention strategies in prevention and mitigation. Options are discussed in further detail in Chapters 6 and 7.

1.3.2 Human health: water safety and food safety

Human and animal sources of infection are similar, with consumption of contaminated food or water a primary means of infection. Humans may acquire infections from contaminated drinking or recreational water, as well as from contaminated food. Food may harbor pathogens due to direct contact with manure or manure-amended soil, via hydrologic transport (irrigation with contaminated water), or through vectors such as insects. Rural drinking water wells can become contaminated in a variety of ways, but because they often serve a single residence, the population impacted is relatively small.

Contamination can be present anywhere en route from the field to the consumer's table. The 2006 outbreak of *E. coli* O157:H7 traced to baby spinach in California serves as an example. Although the primary source of contamination in this outbreak was never definitively identified, several hypotheses were considered, including transport of pathogens from cows grazing in nearby fields and intrusion from wild animals such as feral pigs.

This case emphasizes key research areas, all of which were discussed at the workshop or assessed in the grant studies in some fashion:

1. Proximity to livestock
2. Rangeland runoff
3. Irrigation water
4. Wildlife intrusions
5. Soil amendments

An assessment of human health risk associated with viruses in manure has not been adequately addressed. Most viruses of farm animals, especially the enteric viruses, tend to be fairly host specific. Since there has not been any significant human disease reported with these viruses in the past, there have not been any major studies on the viruses of animals as they relate to human infections. With molecular methods, it has been found that there is the possibility that noroviruses, HEV, and certainly some rotaviruses can be shared between animals and people. Because of the interest in HEV due to its effects on people, there has been significant investment by human health agencies into describing the epidemiology of the disease and cross-transmissibility of types between pigs and people. The noroviruses of animals are now also under scrutiny, and the USDA has committed to determining the scope and magnitude of the human health risks associated with viruses that the agricultural community may need to address.

Gap: The antigenic and genetic relatedness between human and animal enteric viruses needs to be assessed, with special attention to noroviruses and rotaviruses.

One type of study that could aid in determining the zoonotic risks of animal viruses are serosurveys, which are largely lacking. For example, do farm workers on swine farms have higher incidence of occurrence of antibodies to swine noroviruses or rotaviruses? Serosurveys would help determine which organisms are spreading between livestock and people on various farms. Major work, however, will first be required to determine if the assays can differentiate between different viral agents and their subtypes. There have been some serosurveys for the presence of HEV among swine veterinarians,[46] and also for bovine noroviruses among veterinarians versus population controls.[47] Such work, however, is more likely to occur through funding that is outside the purview of the USDA, although the findings are critical to establish the viral zoonotic potential, so we believe that USDA-supported studies are warranted.

Noroviruses and rotaviruses in particular are important causes of gastroenteritis in young children, and similarly, in young animals. These viruses are, for the most part, extremely host-adapted, but may be zoonotic. We recommend that the zoonotic risks of these viruses be further studied. In the interim, despite the low perceived risk, simple management practices could be employed that at the very least would be beneficial for the prevention of *other* infectious diseases, and might help with these as well. For instance, simple separation and treatment of calf and piglet waste may be sufficient to markedly reduce the risk of these pathogens from leaving the farm. Although this would be difficult to manage in cow-calf beef operations, it may be relatively easier to implement on dairy farms through the careful management of calf waste, or, in the case of swine farms, by the separate treatment of nursery waste.

1.3.3 Ecosystem health

Health cannot be examined independent of the interrelationship among the host, the agent, and the environment. Before pathogens reach a human or other animal, they have often interacted in some way with their environment. Fecal-origin material presents a critical source for environmental contamination. The extensive amount of grant-funded work on soil, watershed, and other ecosystem-level dynamics demonstrates the importance of understanding how pathogens enter and move through the environment.

---

[46] Meng XJ, Wiseman B, Elvinger F, et al. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. *J Clin Microbiol* 2002;40:117-22.

[47] Widdowson M-A, Rockx B, Schepp R, et al. Detection of serum antibodies to bovine norovirus in veterinarians and the general population in The Netherlands. *J Med Virol* 2005;76:119-128.

The potential propagation of antibiotic resistance is an excellent example of this interaction. Antibiotic resistance genes may be transported throughout an ecosystem, affecting not only animal and human health directly, but also ecosystem balance. Studying antibiotic resistance in the environment on a large scale is difficult, but some investigations have been able to determine the sources of antibiotic resistant organisms.[48,49] Other work has shown the potential transfer of resistance genes between organisms within the soil.[50] The discussion of the role of agriculture in the propagation of antibiotic resistance could easily be the basis of additional workshops and summary documents. Also, it would probably involve many different agencies besides the USDA. There is not even agreement as to whether humans or animals are the major consumers of antibiotics in the U.S., and whether differing consumption patterns are more or less likely to lead to resistance; this makes rational discourse on the topic very hard to pursue. In addition, the majority of outbreaks in people come from contamination of meat at slaughter rather than from the land application of manure. There is a risk, however, and significant effort is focused on determining the magnitude of that risk.[51] The FDA recently issued a draft document on the use of antibiotics as growth promoters due to growing concern that they might lead to increases in resistant bacteria in humans.[52] The issue requires a determination of the real risks posed by antibiotics in manure and whether there is transmission via the land application of manure to humans or meat products. This will require a very considerable input of effort from many different agencies and research groups with varied research interests. In the meantime, however, we need not be paralyzed back lack of information – we simply must determine if we *can* or *should* delay action until we clarify those risks.

Many questions remain about the spread of antimicrobial-resistant pathogens in the environment, such as whether these organisms outlive species

---

[48] Mthembu MS, Biyela PT, Djarova TG, et al. The use of antibiotic resistance profiling as a means of tracing sources of fecal contamination in source waters. *Water Sci Technol: Water Supply* 2010;10:209-215.

[49] Chee-Sanford JC, Mackie RI, Koike S, et al. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *J Environ Qual* 2009;38:1086-1088.

[50] Heuer H, Kopmann C, Binh CTT, et al. Spreading antibiotic resistance through spread manure: characteristics of a novel plasmid type with low %G+C content. *Environ Microbiol* 2009;11;937-949.

[51] Oliver JD. Recent findings on the viable but nonculturable state in pathogenic bacteria. *FEMS Microbiol Rev* 2010;34:415-425.

[52] United States Food and Drug Administration. *Draft guidance: the judicious use of medically important antimicrobial drugs in food-producing animals.* June 28, 2010.

or strains that are not antimicrobial resistant. We encourage further research in this area.

1.4 Spatial distribution of pathogens

The spatial distribution of pathogens is dependent upon regional geology and hydrology, the chemical composition of the pathogens and the soil particles, the chemistry and physics of the pore water, land use and management, and prevalence and distribution of potential hosts.

Spatial distribution must be considered in the context of environments and scale. For example, in on-farm settings, differences in pathogen shedding by young versus adult animals can contribute to spatial variability. Other on-farm management practices, such as dairy farm feed management, barnyard runoff, and milk parlor management also have significant effect on spatial distribution of pathogens. At the watershed scale, factors contributing to spatial variability include variability in manure application locations and rates; variability in infection levels of different herds; and variability in manure management practices of different farms. On a national scale, state or regional differences in management practices, climate variations, and geographic differences in herd infection rates all contribute to spatial distribution variations.[53]

1.5 The importance of other variables

In the course of the workshop, contaminants other than pathogens, such as antibiotics and hormones, were occasionally discussed. The request, however, from the USDA was to present a synthesis of what we know about pathogens in watersheds. So that is what the workshop primarily addressed, and what is presented here.

We know, of course, that pathogens are only part of the story. Ideally, any guidelines, regulations, or laws promulgated to mitigate watershed contamination will take a holistic approach that encompasses reasonable measures for all pollutants of concern.

We would be remiss, however, not to take this opportunity to clarify a misconception about hormones as a contaminant. Hormones, like pathogens,

---

[53] Due to the often low number of pathogens in protocols for monitoring spatial variability, it may be useful to monitor for coliphages to help determine the spatial distribution of pathogens.

can be excreted in feces. Many in the meat and milk industries have been responsive to public pressure not to administer growth hormones to their animals, a controversial decision as the FDA has determined that the consumption of animal growth hormones does not negatively impact human health. Bovine somatotropin (bST) is a naturally-occurring hormone produced by the pituitary gland that functions in bovids as a growth promoter, as a lactation promoter in cows, and in homeorhesis (essentially, normal bodily functions). A recombinant (synthetic) form (rbST) is administered to animals in many operations to increase milk or meat production while reducing nutrient inputs per milk yield, water use, and waste outputs. Administration of rbST fosters efficiency, leading to more end-product with fewer inputs. bST and rbST, which are peptide hormones, should not be confused with other types of hormones that may affect human health, such as steroid hormones produced naturally and excreted by production animals, that may reach the consumer through food or contaminated water. For elaboration on this issue, please see the Appendix for references.

Concluding Remarks and Key Points

Watersheds provide water for irrigation, drinking water, industrial water, recreational opportunities, and ecosystem functions. But their very nature to drain the land that defines them makes them susceptible to myriad land-based inputs, including contaminants. Bacteria, viruses, and protozoa are all known pathogenic contaminants of watersheds. In order to prevent their spread and mitigate their potential impacts, we must:

1. Better understand:
    a. pathogen sources (i.e., human or other animal);
    b. post-shed behavior of pathogens;
    c. pathogen zoonotic capabilities, evolution and emergence from a host reservoir into other species;
    d. how environmental factors affect pathogen fate;
2. Develop and assess better indicators for human and other animal sources of feces, particularly those that can distinguish between human and other animal sources.

2

Chapter 2: Survival and Reservoirs

Survival capacity of a pathogen is specific to both the organism in question and the environment in which it finds itself. Pathogens vary widely in their ability to survive in a given environment. Some have special properties that allow for greater longevity, such as cyst formation. What factors enhance the survivability of pathogens in an agricultural environment? We know a fair amount about the number of days that pathogens survive, but less about the mechanisms by which they do so.

Half-lives of pathogens are dependent on both the nature of the pathogens and environmental variables. Some half-lives have been determined in the laboratory but not in the field. More realistic studies of survival that mimic environmental conditions would be valuable. Quantitative information is needed on die-off of pathogens to develop predictive models for water quality and in risk assessment.

Gap: Field-based, or highly realistic laboratory-based, studies of pathogen survival would add value to the understanding of survival and formation of nidi of infection within different environmental conditions. ("Nidi" (plural of nidus) are here defined as those places where a pathogen originates, is fostered, or develops; specifically, the points of origin or focus of an infection or disease process. These environmental nidi are distinct from "animal reservoirs," an animal host or passive carrier of a pathogen.)

Available assessments of pathogen survival represent the aggregation or averaging of sets of case studies. There is little understanding of how survival varies with specific material properties (composition of soils and sediments, etc). There is also little understanding of spatial variability of survival within natural systems, and the effect of the freeze-thaw impact on survivability of cells or aggregates. Many California outbreaks have occurred in the late summer to early fall. Is this a result of seasonality of shedding? Or do pathogens oversummer in the soil? Do they grow and increase in numbers after rainfall events in the soil? Most existing reviews are specific to one type of environment; substantial spatial heterogeneity in pathogen survival in different environments or environmental niches should be expected.[54]

Gap: Comprehensive assessments and comparisons of survival in diverse media (e.g., soil, water, manure) are needed for understudied pathogens, including *Blastocystis* sp. and microsporidia, about which little is known. The survival dynamics of even those pathogens that have been extensively studied, such as *E. coli* O157: H7 and rotavirus, are still not entirely elucidated.

[Note: Since the introduction of low-temperature sterilization by Louis Pasteur, we have learned a great deal about how to inactivate pathogens and about how well they survive. At the same time, detection levels have markedly improved in recent years, so we are better able to identify pathogens and their sources. Thus, the question that always arises both in disinfection and survival studies is how much one can apply from the general to the specific. Is the survival of the *E. coli* O157:H7 any different in the environment than another *E. coli* lacking the virulence trait? Is the difference significant in terms of survival in the environment, even if it is significant in different hosts? The same holds for viruses: is understanding the survival of a generic norovirus sufficient, or do we need information on specific isolates from around the world? Can we extrapolate from non-pathogenic forms in the laboratory to pathogenic forms isolated from an outbreak? The same issues have been raised with the survival of *Cryptosporidium*. For most organisms, a very comprehensive collection of survival data has been assembled with different strains and species under different scenarios. It would be worthwhile to develop a database that would collate all this information for the different agents from around the world, although concerns of bioterrorism might necessitate classification, thereby limiting its usefulness.]

Gap: The viability of enteric viruses when sequestered in manure, soil, or organic materials has not been well studied.

---

[54] It is interesting to note that many organisms are most prevalent environmentally in late summer in many areas of the U.S. It is not clear if this is strictly a temperature effect or involves more complex ecological interactions (e.g., related to algal blooms, vegetation growth, or changes in water flow – and this could be either higher flows (flooding → export) or lower flows (less water → concentration)). It is hard to identify unique environmental factors or to discriminate regional trends for survival of the relatively robust environmental pathogens because the ecological interactions are very complex in most environmental reservoirs.

Great strides have been made in understanding survival and die-off in specific conditions, and average behavior is now understood in soil and water, but the distribution of survival in complex environmental nidi is still not comprehensively elucidated. Single rate coefficients do not capture the full complexity of diverse microbial populations. Also, recent research has demonstrated the enhanced persistence of subpopulations of pathogenic organisms, but the mechanism is not well understood. Exponential die-off with a constant inactivation rate or half-life provides a general description, but does not describe the preferential long-term survival of some subpopulations. Limits of detection often prevent discrimination of long-persistence subpopulations, viable but non-culturable subpopulations (VBNC), etc. VBNC organisms in particular pose a challenge to optimal assessment of samples. Although it is believed that long-term persistence of subpopulations is important for disease transmission, and perhaps for the persistence of antibiotic-resistant subpopulations, we do not comprehensively know how important it is for each agent of interest.

Although survivability is important, it is not the only factor that leads to outbreaks. Survival merely increases the likelihood that the pathogen can be transported to a water body or vegetable crop where human exposure may occur. Pathogen concentration is equally important in determining if disease results from exposure, but the survival rate influences both the concentration of viable organisms and the duration of risk. This is one of the factors that makes it difficult to predict outbreak risks.

Gap: The biological mechanisms allowing bacteria to enter a persistent state are not well understood. Furthermore, how bacteria in persistent states (e.g., VBNC) regain activity is not understood.

2.1 Bacteria survival

Bacteria associate with soil and sediment particles. The nature of the association is a function of particle mineralogy, presence of organic matter, and solution chemistry. Aside from phenotypic traits of the pathogen itself, factors that affect the survivability of bacteria (and pathogens generally) in sediment and soil include:

- pH
- salinity

- moisture content
- temperature
- aeration
- plant root environments/rhizosphere interactions
- substrate characteristics, including nutrient availability
- ultraviolet light exposure
- biofilm production
- redox potential
- predation
- access to host
- indigenous microbial community dynamics, including competition and synergistic relationships

Generally speaking, bacterial pathogens can persist for several months to more than a year in the environment. Initial growth is followed by a rapid decay. Decreasing rates of die-off over time, leading to long-term persistence of a relatively small number of organisms, is then observed for many bacteria, leaving a population known as the "super-surviving fraction." In warm tropical/sub-tropical waters, indicators such as coliforms and *E. coli* grow naturally and cannot be used as indicators of fecal contamination. We need to better understand this phenomenon, especially the ways that climate change may affect the use of these indicators in temperate climates.

Although it poses a considerable challenge, the study of pathogen survival should emphasize actual environmental conditions. This can be done through the use of sentinel chambers, lysimeters, rainfall simulation studies, and realistically complex materials in laboratory studies, as we see in many of the grant-funded works depicted in this report.

Yan[55] studied survival of *E. coli* on soil particle surfaces in three Hawaiian watersheds. The study, which is still underway, has thus far determined that while the abundance of *E. coli* in soil does not correlate with land use, the abundance of *E. coli* in water does show a significant level of correlation with land use. By obtaining and analyzing *E. coli* using DNA fingerprinting techniques, Yan found tremendous genotypic diversities in the soil and water of the watersheds. These data are being prepared for publication. Yan is currently developing a high throughput technique for screening the soil *E. coli* isolates for their survival on particle surfaces under various environmental stresses.

---

[55] Yan T. Survival of *Escherichia coli* on soil particle surface in Opaeula watershed of Oahu, Hawaii. Contract no.: 2009-35102-05212.

Subsequent genetic analysis will be carried out to identify the underlying mechanisms of the *E. coli* super-survivors in tropical soil.

Dou, Rankin, and Aceto[56] have examined survival and fate of bacteria in manure and manured soils, focusing on *E. coli* O157:H7 and *Salmonella* serovar Newport. Sentinel chambers were used to monitor the survival of the pathogens under field conditions, where pure cultures of the bacteria were inoculated into manure or soils. Log reduction time in dairy effluent lagoon system (at seven to nine days) was approximately half that of the field soil samples. The authors posit that competition or antagonistic interactions between the introduced pathogens and native microorganisms in the manure was probably a contributing factor. In summer field soil sentinel trials, the two bacteria had different survival patterns, with the *Salmonella* rate of deactivation lower than that of *E. coli*, perhaps due to lower tolerance among *E. coli* for the low soil moistures typical of the growing season.

Unpublished data demonstrate that three *Salmonella* serovars (Dublin, Newport, and Typhimurium) declined linearly to detection limit at similar rates, although their maximum survival (measured by the most probable number [MPN] procedure) differed: *S*. Dublin was not detected after 98 days, *S*. Newport after 160 days, and *S*. Typhimurium after 176 days. Strains of *E. coli* O157 Shiga toxin positive (stx+) had similar deactivation rate in the soil samples as *E. coli* O157 stx-; however, *E. coli* O157 stx+ and streptomycin resistant had a lower deactivation rate and survived longer. After initial deactivation, all pathogens persisted at low concentrations for months following introduction into the agroecosystem. An additional interesting finding was the compost pile test: the bacteria were killed within 18 hours of installation (along with native compost microbes), presumably by the high temperatures generated in the compost (up to 64° C).

2.2 Virus survival

As for any pathogen, virus survival is dependent on a host of factors:

- temperature – longer survival at lower temperatures or in a frozen state
- pH – survival from pH 3.0 to 10.0, with optimum level at pH 7.0 to 8.0, depending on the virus

---

[56] Dou Z, Rankin S, Aceto, H. Survival and transport of *E. coli* 0157:H7 and *Salmonella* Newport in manure and manured soils. Contract no.: 2007-35102-18243.

- organic matter – longer survival in sewage-contaminated waters, depending on the virus
- turbidity – longer survival in turbid waters
- sediments – longer survival in sediments

Enteric viruses can generally persist in the environment much longer than enteric bacterial pathogens, and under low temperature may persist for years. They are more resistant to UV light and elevated temperatures than enteric bacteria and protozoa. Temperature has been shown to be useful for predicting viral survival times in water.

Jin, Sims, and Kniel[57] evaluated virus survival in biosolids and manure. The survival of Ad41 was measured based on genome stability and infectivity in different types of manure and three types of biosolids, including pelletized poultry litter (PL), alum-treated poultry litter (AL), raw poultry litter (RPL), liquid dairy manure (DM), swine manure (SM), and three types of biosolids 1, 2, 3.[58] Overall, Ad41 viral genomes were found to be stable at both 20°C and 4°C and there was no significant loss of viral DNA after 60 days in PL, AL, biosolids type 1, and DM. However, infectivity was lost almost immediately in high pH biosolids type 2 and 3, and infectivity decreased quickly in DM. Ad41 had ~1.9 log loss of infectivity after added in SM and biosolids type 1 at day 0, and estimated T90 was 12.5 and 28.6 days for biosolids type 1, and 19.1 and 51.0 days for SM at 20°C and 4°C, respectively. Ad41 maintained infectivity in all three poultry litters, and after 60 days incubation, there were significantly more infectious virus in PL, AL, and RPL than biosolids 1, SM, and DM at 20°C.

In addition, the survival and infectivity of Murine norovirus-1 (MNV, a surrogate for human noroviruses) and hepatitis A virus (HAV) was evaluated in biosolids and manure samples.[59] Three types of biosolids were obtained from wastewater treatment plants in Maryland, and liquid dairy manure and three types of poultry litter were obtained from farms in Delaware. MNV and HAV were inoculated into the samples and kept at 20°C and 4°C for up to 60 days, and then extracted and analyzed via RT-qPCR. Survival varied with the substrate. For example, MNV genomes were stable in solid manure and some biosolids, but degraded immediately in high pH biosolids and were relatively unstable in liquid

---

[57] Jin Y, Sims JT, Kniel K. Effect of land application of wastes on the fate and transport of pathogens in soil. Contract no.: 2006-35102-17405.

[58] Wei J, Jin Y, Sims JT, et al. Survival of human Adenovirus 41 in land applied manure and biosolids. *Food Environ Virol* 2009;1:148-154.

[59] Wei J, Jin Y, Sims JT, et al. Survival of murine norovirus and Hepatitis A virus in different types of manure and biosolids. *Foodborne Pathog Dis* 2010. doi:10.1089=fpd.2009.0490. (In press)

manures. HAV also degraded immediately in high pH biosolids at time zero. Overall, both viruses were inactivated rapidly in alkaline pH biosolids. Infectivity also varied with substrate and virus. For example, in terms of MNV infectivity, there was no significant difference among the poultry litters and swine manure at either 20°C or 40°C after 60 days. HAV stored in swine manure and raw poultry litter, however, had significantly higher infectivity levels than HAV stored in alum-treated poultry litter. In summary, both viruses were inactivated most rapidly in lime-stabilized biosolids with alkaline pH, and were unstable in liquid dairy manure. Alum added to poultry litter inactivated some HAV at both temperatures but had no effect on MNV. Overall, the results suggest that solid manure and biosolids with low water activity and neutral pH have the greatest risk for contaminating soils.

These studies emphasize the highly complex nature of viral survival dynamics. Another effort worth mentioning in this context was an examination the effects of conventional and five new candidate animal waste treatment technologies on the detection and viability of in situ porcine viral enteropathogens (rotavirus-A, B, C, sapovirus and norovirus) in swine manure.[60] Procedures were developed for viral RNA extraction and testing and reduction of PCR inhibitors in the manure samples, followed by RT-PCR and nested PCR assays. The viruses, except rotavirus-B were present in pre-treatment manure samples of at least three of five farms evaluated. After the various candidate swine waste treatments, however, only rotavirus-A and rotavirus-C RNA (but not infectious virus) were detected. The findings suggest that widespread implementation of the selected environmentally superior waste treatment technologies could reduce or eliminate viable enteric viral pathogens.

2.3 Protozoa survival

Under moist, cool conditions, *Cryptosporidium* oocysts can survive for many months, and *Giardia* cysts can survive for several weeks. *Toxoplasma gondii* can survive even longer: over a year in soil, and for longer periods in water at cool temperatures. *Balantidium coli* cysts are probably capable of surviving well in cool or cold water without significant bacterial contamination, but exact durations of survival are as yet unknown. *Balantidium* cysts are relatively heavy compared to other protozoa and are more likely to collect with sediments than to stay suspended like the cysts of *Giardia* or the oocysts of

---

[60] Constantini VP, Azevedo AC, Li X, et al. Effects of different animal waste treatment technologies on detection and viability of porcine enteric viruses. *Appl Environ Microbiol* 2007;73:5284-5291.

Cryptosporidium. Desiccation is highly detrimental to oocyst and cyst survival in most protozoa. *Blastocystis* sp. do not survive well outside the host, surviving only a few hours at room temperature or at refrigerator temperatures in feces or media. Microsporidia, being spore-forming organisms, also have the capacity to persist environmentally for several days to weeks, but great variability exists among the many genera of microsporidia, and the exact duration of survival in different environments is again unknown.

Gap: Although some survival dynamics are known, there is an overall deficiency of data for *B. coli*, *Blastocystis* sp., and microsporidia.

2.4 Modeling survivability

Multiple factors influence survival in the environment, such as the presence or absence of environmentally protective features (cysts, walls, or other protective coatings), ability to resist desiccation, sunlight exposure, mechanical disruption, reduced metabolic state, food storage vacuoles, and the ability to utilize nutrients from the environment. Temperature and sunlight exposure have been found to be the most useful for predicting pathogen survival in the water environment. Pathogen interaction with other factors is less understood and more difficult to predict.

Survival is not a simple linear process. It can be modeled using a first-order decay model ($\lambda=\lambda_o\exp(-\alpha t)$), where survival ($\lambda$) is governed by time (t) and an experimentally determined inactivation coefficient (α).[61] The exponential relationship is often found to adequately describe survivability, but is not optimal in all cases. The first-order decay model has been used in watershed scale modeling of pathogen transport and survival. The Gauss-Newton model may be a viable alternative to describe survivability.

Gap: The scientific community lacks surrogates that would allow field testing of survival models, such as the use of bacteriophages for enteric viruses. The surrogates would first have to be benchmarked against the pathogens of interest prior to development.

In general, better models are needed. We need to understand both the biological factors that contribute to long-term survival (i.e., how exactly are subpopulations different?) and also if or how these organisms behave differently

---

[61] Papchepsky YA, Sadeghi AM, Bradford SA, et al. Transport and fate of manure-borne pathogens: modeling perspective. *Agric Water Manag* 2006;86:81-92.

in the environment (i.e., is long survival their only unique feature relative to the whole population? Or do they behave differently in other ways as well, such as in motility/mobility, stickiness/attachment, or virulence? What role is climate change playing?

Gap: The scientific community should develop models of pathogen survival that incorporate factors contributing to the long-term fate of super-surviving subpopulations, and the effects of climate change.

2.5 Environmental nidi

Nidi that allow extended survival or growth of pathogens in the soil and aquatic environments are known, but the significance of these microenvironments to pathogen dynamics at the watershed scale is largely unknown. Storage of animal and human waste may support survival for differing periods of time, as may sediments (e.g., in streambeds, lakes, and water reservoirs) and soil and water distribution systems. Storage containers can be decontaminated, but it is impractical and environmentally harmful to disinfect soils, sediments, and water bodies. Stream sediment, aquatic vegetation, algal mats, periphyton, and biofilms may harbor microbes, as well as suspended particles (complex aggregates) in bodies of water such as lakes or estuaries.

Robust, opportunistic pathogens can potentially colonize and reproduce in some environmental reservoirs. This is definitely a concern for *Legionella* and potentially for *E. coli*, *Salmonella*, and *Campylobacter*. The potential for colonization of environmental reservoirs should be assessed along with die-off.

Biofilm growth on soil and sediment particle surfaces, irrigation systems, and water distribution systems can also provide a survival nidus. Biofilms provide microhabitats for pathogens, in some cases enabling survival. Biofilms are also known to greatly protect resident organisms from biocides and other chemical stressors, making it extremely difficult to eliminate biofilms from drinking water supplies by means of chlorine or other disinfectants.

2.6 Retention mechanisms

Great progress has been made in understanding pathogen and particle aggregation and filtration mechanisms under laboratory conditions. Natural particles are very diverse – soil and sediment particles are often complex aggregates that have a high degree of heterogeneity in composition, size, shape, density, porosity, etc. Organisms are also very diverse, and can interact with natural background materials in complex ways. Many organisms have complicated structure and mechanisms for regulation of their surface properties, motility, etc. Their interactions with environmental interfaces are therefore complex. Again, progress has been made recently with some high-priority pathogens under laboratory conditions, such as cellular attachment and biofilm formation on simple materials, but it is still not clear what processes are most important in complex natural systems.

A study by Bryant[62] sought insight into the mechanism behind the retention of pathogens in unsaturated soils. Observations made from micromodel experiments suggest that stability of colloid retention (used to simulate bacterial retention) at interfaces is controlled by a balance of colloidal, drag, and surface tension forces. The study also provided insight into the dynamic, local nature of colloid retention. Not all air-water-solid contact lines are created equal, and any model that treats them as functionally equivalent in terms of their ability to retain colloids will not be predictive of field behavior. Examinations of breakthrough time and the concentration of colloids at steady state during both infiltration and drainage, combined with the computed length of contact line and interfacial areas for the analogous geometry, is helping the researchers to identify whether contact line or interfaces are the controlling mechanism for pathogen retention.

Fecal pathogens are always introduced with a complex matrix, and then interact with and redistribute on natural background materials (soils, suspended sediments, streambed sediments). Interactions between pathogens, soils, sediments, fecal matter, and organic matter are not well understood, but are expected to play an extremely important role in pathogen mobility, transport, and survival. Steady processes are much better understood than dynamic ones. Natural systems have many perturbations and are highly transient over a wide range of time scales (e.g., diurnal, seasonal) and the potential for episodic transport and release of pathogens is not well understood. This is important because of known linkages between outbreaks and unusual conditions or perturbations, such as floods, and the general importance of variations in light, temperature, and water chemistry to microorganisms. Improved knowledge of the response of pathogens to perturbations in temperature and water inputs is particularly important because of climate change. Not only is mean temperature expected to increase, but this will also shift regional rainfall patterns and

---

[62] Bryant SL. Quantifying the mechanisms of pathogen retention in unsaturated soils. Contract no.: 2007-35102-18162.

produce increased variability in runoff, streamflow, and other environmental flows that can support and/or transport pathogens.

Gap: Interactions between pathogens, soils, sediments, fecal matter, and organic matter are not well understood.

2.7 Community microbial ecology

Metagenomics tools could improve understanding of pathogen reservoirs. Genetic variability in pathogen populations clearly occurs, but how to comprehensively identify environmental reservoirs of pathogens is unresolved. For most pathogens, there are extensive databases for clinical samples, but not for environmental samples. Information is lacking on genetic variability in diverse environmental media. Similarly, differences in environmental transmission and survival of sub-populations have not been linked to genotypic and phenotypic variability in the population. Environmental work to date has primarily been in open-water environments, and not in heterogeneous environments such as soils and sediments.

On a final note, it is emphasized that ecological interactions are very important for pathogen persistence in soils, aquatic environments, and constructed wetlands used to treat agricultural runoff and feedlot effluent. In general, ecological interactions within microbial communities are not understood well enough to make good predictions for most pathogens.

Concluding Remarks and Key Points

Survival kinetics are complex processes dependent on a range of factors, both inherent to the host and dependent on the characteristics of its environment. Science is not yet at the point at which it understands the survival potentials of all pathogens of concern under all scenarios. The same holds true for niches where pathogens survive until their further transmission.

Grant work discussed in this chapter has led to many valuable findings: the relatively high contamination risk of solid manure and biosolids with low water activity and neutral pH; the perceived competitive interactions between pathogens that led to differential survival times; and the development of a technique for screening soil *E. coli* isolates for survival under different environmental stressors;

It remains incumbent on the scientific community to:

1. Better understand pathogen stability and viability in manure, soil, and water under different environmental treatment and disinfection conditions;
2. Develop better models for the prediction of pathogen survival, especially those that would incorporate:
    a. factors contributing to the long survival of select subpopulations;
    b. the effects of climate change;
3. Identify and characterize pathogen growth and survival in environmental microhabitats, such as sediment or biofilms, where knowledge is limited; and
4. Develop improved mathematical descriptions of growth, survival, and retention for modeling pathogen behavior at the field and watershed scales, including the effects of complex environmental habitats (spatial patterns) and natural variability in water inputs, flow, light, and temperature (temporal dynamics).

3

Chapter 3: Pathogen Transport and Fate

How pathogens spread from their source of origin into other parts of a watershed, and ultimately to people, is a topic of considerable interest. One of the resounding, underlying questions of the workshop was: what are the dynamics of pathogen fate and transport in rural and agricultural areas? We understand that hydrologic transport is the primary means of pathogen movement in the environment. But natural flow systems are dynamic, complicated to model, and challenging to predict. Nevertheless, understanding how these systems function can lead to opportunities for preventing or mitigating disease transmission.

Schnabel, Barnes, and Toniolo[63] studied the interactions between pathogens and soil particulates in spring melt water, a water supply that represents a major proportion of the annual pathogen loading in many cold region waterways. Lysimeters were constructed to capture moisture flowing from the melting snow from the sampling site in Alaska. The lysimeters contained a layer of manure-laden soil on top of a basal layer of manure-free soil, which served as a drainage layer to capture infiltrated moisture and direct it towards a collection container. Samples were then tested for total coliforms, *E. coli,* and *Enterococcus*. Preliminary results indicate that differential survival and transport characteristics of the pathogen indicator organisms studied in snowmelt lead to a significant disparity between the fates of the organisms.

---

[63] Schnabel WE, Barnes DL, Toniolo HA. Partitioning behavior of pathogen indicator microbes in springtime meltwater. Contract no.: 2008-35102-19215.

The message here is that transport is highly variable. Furthermore, although the transport of fecal indicator microorganisms in model systems has been studied, pathogen transport in the environment has received less attention and it is not fully understood how differences between indicator bacteria and pathogens are affected by transport processes. It is challenging to transfer information from model systems to complex environmental systems. Key issues include scaling laboratory or plot studies up to large farms and watersheds, and understanding how spatial heterogeneity affects transport. We know that heterogeneity leads to opportunity for small quantities of suspended material to travel much faster than average through preferential flow paths, but the complete role of subsurface heterogeneity (soil and groundwater) is still unclear.

Transport-retention processes function at several spatial scales:

1. Nanoscale: uptake of material into pathogens, and interactions between pathogen surfaces and environmental surfaces.
2. Microscale: pathogen movement within soil pore spaces and adsorption onto soil particles, influenced by processes such as electrostatic interactions, biofilm growth, soil porosity, and physical filtration.
3. Field scale: storage and migration on the land surface and soils, influenced by fencing, manure and compost storage, manure incorporation, and impoundment integrity. Mulching, contouring, terracing, grassed waterways, cover crops, conservation drainage, and buffer strips to increase infiltration and reduce runoff and erosion also have their effects at this level.
4. Watershed (meso-landscape) scale: storage and migration at the scale of multiple farms and multiple environmental reservoirs, a level at which water drainage networks, windborne transmission of aerosols, and vehicle transport all play a role.

Superimposed on each scale are drivers of variability such as seasonal temperatures, regional differences in soil, or source conditions. From the workshop we gained useful insights concerning the processes acting at each of these different spatial scales and also highlighted gaps in knowledge.

We understand transport processes much better than we did a decade ago, as the breadth of transport studies in this chapter demonstrates. Nonetheless, there is still a gap in our ability to predict the dynamics of pathogens in what are extraordinarily complex environmental systems. Importantly, we do not understand pathogen behavior well enough in a comprehensive fashion to thoroughly evaluate risks. Many grant projects were directly or indirectly related to transport. The studies ranged from investigations of fundamental understanding of pathogen movement, to highly specific studies of transport mechanisms.

Gap: We do not understand pathogen behavior well enough in a comprehensive fashion to thoroughly evaluate risks.

3.1 Factors that affect transport

In general, pathogen transport is controlled by bulk fluid flow, mixing processes (diffusion/dispersion), and retention processes (e.g., attachment to surfaces/particles). There are *many* potential transport pathways, such as runoff, subsurface drainage, preferential flow through macropores, stream and river networks, roadside ditches, estuaries, ponds, and many more. Furthermore, many factors may affect transport, including soil type and characteristics, rainfall intensity, snowmelt intensity, topography, and, importantly, manure and land management.

Transmission pathways, transport rates, mobilization-immobilization processes, and survival are all very complex, highly heterogeneous phenomena that are often unsteady over a wide range of scales. In general, we understand the significance of single factors in isolation, but not their interaction in complex natural systems. Similarly, we understand behavior of relatively small and homogeneous systems, and also general trends at large scales, but we cannot link processes across scales in order to assess overall transport pathways or adequately predict risks. This chapter addresses what we do know about transport, and where the gaps in our knowledge are.

Soils may be the most effective location for immobilization of pathogens in the environment. Yet the characteristics of a given soil may make it, or the pathogens contained in it, transportable due to runoff and leaching. Soil texture, binding capacity, dusting, slope, distance to stream, depth to groundwater, level of surface application versus incorporation, tile or roadside ditch drainage and macropores, filter strips, and erosion controls such as ground covers all help determine how efficient that transport will be. Export of pathogens from farm environments is intimately linked to soil moisture status. Export may occur as a result of overland flow (runoff) or movement in groundwater following infiltration into soils, movement of soils by erosion, or direct transport of animal feces or manure. Factors that affect transport in the subsurface include filtration/straining, soil texture, surface charge of soils and bacteria, soil chemistry, attachment to particulates, and surface application versus incorporation of manure. Factors that affect overland migration of pathogens include hydrology (overland flow versus infiltration), soil erosion, attachment to particulates, precipitation history, cover crops and other forms of vegetative cover, surface application versus incorporation of manure, and distance to water body. Surface and subsurface transport typically vary strongly with climate and season owing to pronounced differences in water inputs and variation of many factors with temperature.

There is a need for further investigation of runoff events. Rapid conduits for the export of pathogens from animal feces in pasture and rangeland or manure-amended fields to surface water bodies include tile drainage, grassed waterways, roadside ditches, and other drainage structures. Studies have documented that networks of roadside ditches efficiently transport high loads of *E. coli* from manure-spread fields to drinking water supplies located many miles downstream in just a few hours.[64] Significant reductions in export of fecal bacteria could be achieved through concerted efforts to design and implement pathogen-specific BMPs for these critical control points.

Several landscape management practices exist to restrict manure export, including vegetative and riparian buffers, infiltration basins, and wetlands, among others. These practices have been shown to reduce bacterial transport by one to two log orders, but are all subject to failure when the soil is saturated and extensive surface runoff occurs. Precipitation-related infiltration may dilute soil pore water, causing detachment and increased transport of pathogens in the subsurface. The concentration of fecal bacteria in impacted streams and rivers is directly proportional to the intensity of overland flow and stream response. Studies have shown that both the flux and concentrations of fecal bacteria increase with hydraulic flux (hydrograph peaking) in manure-impacted streams.[65] Bacterial concentrations exhibit long-term tailing behavior as stream waters recede.

Relatively few studies have examined runoff of bacterial pathogens and fecal indicator bacteria following manure application to frozen or snow/ice covered ground. (The Schnabel, Barnes, and Toniolo work referenced at the start of this chapter, however, presents a good example.) There is evidence from

---

[64] Falbo K, Schneider RL, Buckley D, et al. Submitted to *J Environ Qual* August 2010.

[65] Lewis DJ, Atwill ER, Lennox MS, et al. Linking on-farm dairy management practices to storm-flow fecal coliform loading for California coastal watersheds. *Environ Monit Assess* 2005;107:407-425.

the few studies that exist that loading to nearby surface waters during thawing cycles may be in excess of loads that would otherwise be generated from more conventional (pre-planting or post-harvest) manure application. These scenarios may require special consideration when selecting landscape management practices to limit the export of pathogens from land-applied manures to surface water bodies.

Drizo, Gouli, Gouli, et al. looked at *E. coli* and *Cryptosporidium* occurrence, transport, reduction, and fate in point and non-point pollution sources at the University of Vermont dairy farm and data are being prepared for publication.[66,67] Water samples were collected at seven sampling points: three from different point sources (manure tank, splitter tank flume, dairy tank), and three from non-point sources (feedbunks runoff, east and west side), and Potash brook at the dairy at each rain and snowmelt event during 20 months. Of the investigated sources, dairy tank had the highest average *E. coli* concentrations (772 x 10^4 *E. coli* per mL) followed by feedbunk runoff (259.14 x 10^4 *E. coli* per mL) and splitter tank flume consisting of the mixed barnyard runoff and dairy effluent (210.14 x 10^4 *E. coli* per mL). Potash brook, which is classified as impaired stream in Vermont, had an average *E. coli* concentration of 9.1 x 10^4 *E. coli* per mL. Seasonal variation analysis showed that the highest *E. coli* concentrations were observed during summer, reaching 3096.55 x 10^4 (dairy tank) and 986.80 x 10^4 (feed runoff) *E. coli* per mL. Dairy tank also had very high *E. coli* concentrations (911.78 x 10^4 *E. coli* per mL) in winter and the lowest in spring (14.45 x 10^4 *E. coli* per mL). Feedbunks runoff was the second largest source of *E. coli*, having concentrations of nearly 100 x 10^4 *E. coli* per mL in both spring and summer and nearly 50 (46. 07 x 10^4 *E. coli* per mL) during winter. Drizo and co-researchers have developed a simple, low-cost, steel slag-based filter system for phosphorus and *E. coli* reduction from surface (open) channels, which is currently showing promising results in testing for tile drainage treatment.

Experimental BMPs may include such efforts as “conservation drainage,” which, while mostly used for nutrient control, is an up-and-coming BMP and may have implications for pathogens. This type of drainage tends to hold soil water in the system for long periods of time in the growing season. Another promising

---

[66] Drizo A, Gouli V. Escherichia coli and Cryptosporidium occurrence, transport, fate and reduction from Vermont dairy farm point and non-point pollution sources. Contract no.: 2008-35102-19222.

[67] Drizo A, Gouli V, Twohig E. et al. *Escherichia coli* and *Cryptosporidium* occurrence, transport, fate and reduction from Vermont dairy farm point and non point pollution sources. Final grant report to the USDA National Institute of Food and Agriculture (NIFA), August 2010.

experimental BMP is the installation of filters (slag or carbon) into tile drainage lines to reduce pathogen loading from tile-drain discharge. However, a 1987 report on drainage status in the U.S. states that "drainage is the most extensive soil and water management activity in agriculture."[68] Thus, because a vast amount of the farmland in the U.S. contains tile drains, installing filters and their routine upkeep or replacement would require a major commitment of resources.

Gap: The coupling between flow and sediment is not well understood. The relative role of direct transmission of pathogens in water vs. release and transport of pathogens associated with soils and sediments is not well understood.

Retention variables include soil infiltration, adsorption, predation, vegetative cover, lack of soil moisture, pH, soil redox potential, and temperature. Pathogen retention within the environment, followed by re-release into the flow, may be the primary items of impact of both groundwater and surface water flows. There is little understanding of how transport, retention, and survival vary for particular populations or subpopulations of pathogens and with specific material properties (e.g., composition of soils and sediments). There is substantial spatial heterogeneity in transport and survival in soils, sediments, etc. Even something as ostensibly simple as the relationship between flow and sediment loads is not yet fully elucidated. One of the challenges to overcoming this gap is in finding resources for flow monitoring so the effects of flow on sediment and pathogen loads can be properly determined, , and the state of pathogens in transport can be identified (e.g., freely suspended vs. associated with suspended sediments or biofilm cell clusters).

Soils and sediments have been identified as likely nidi for pathogens, and many deposition and resuspension mechanisms are known. Yet interactions with sediments in aquatic systems (streambed sediments, ponds/reservoirs, estuaries, etc.) are not well understood. Sediments in roadside ditches act as a reservoir of viable *E. coli* long after storm events and may be subject to resuspension and later transport to downstream drinking water.[69] Remaining major uncertainties in this area include:

- mobility/transport/resuspension of pathogens in complex natural water bodies under highly variable flow conditions;

---

[68] GA Pavelis, ed. *Farm drainage in the United States: history, status, and prospects*. USDA-Economic Research Service. Misc. Pub. No. 1455. 1987. Washington, DC: U.S. Government Printing Office.

[69] Falbo K, Schneider RL, Buckley D, et al. Submitted to *J Environ Qual* August 2010.

- microbial ecology of pathogens in benthic/hyporheic communities; and
- integration/upscaling to watershed-scale releases (linking sources to effluents, and timing of releases).

Harter[70] is seeking to understand similarities and differences in the occurrence, fate, and transport of key zoonotic pathogens and indicator organisms in soil water, groundwater, and surface water. An additional goal is to provide a conceptual framework for the quantitative assessment of pathogen transport in the surface water-groundwater continuum. The ongoing research is helping to develop a new understanding of pathogen retention and transport behavior in soils, groundwater, runoff, and agricultural streams, and has also produced valuable data on the occurrence and transport of pathogens at the whole-farm scale and on confined animal facilities.

3.2 Bacteria transport

Much research suggests that *E. coli* and other bacteria are predominantly transported attached to sediment. Various factors leading to increased sediment erosion in the landscape, such as farm fields left exposed to early snowmelt and runoff, or roadside ditches scraped and left unvegetated, can therefore favor pathogen release. Climatic factors, such as increased high intensity rainfall events, are also more likely to lead to erosive runoff. However, more research is needed to identify sediment adsorption characteristics of different species of pathogens, as there are conflicting data concerning their transport in groundwater versus surface water and with peak flush of runoff versus later in hydrograph.

Dou, Rankin, and Aceto[71] have examined transport through and retention in the soil profile of *Salmonella enterica* serovar Newport and fluorescent protein-labeled *E. coli* O157:H7. Soil columns of 10 cm diameter and 15 cm depth were collected from the field. Soil variables were undisturbed cores with sod cover versus tilled, or silt loam versus sandy loam texture. Pathogens were inoculated into dairy manure and applied to the surfaces of the cores. Simulated rainfall was applied periodically over fourteen days with three different application patterns. Leachate samples, soil increments, and manure samples were collected and enumerated for concentrations of *Salmonella* and pathogenic

---

[70] Harter T. Human health risks from animal agriculture: comparative analysis of the transmission of multiple zoonotic pathogens in mixed-use agriculture. Contract no.: 2008-35102-18733.

[71] Dou Z, Rankin S, Aceto, H. Survival and transport of *E. coli* 0157:H7 and *Salmonella* Newport in manure and manured soils. Contract no.: 2007-35102-18243.

E. coli. Ongoing analysis of the data is showing significant differences in pathogen leaching and soil retention behavior as a function of both tillage and soil texture. *Salmonella* losses in leachate were not different from those of *E. coli* O157:H7 in the silt loam soil, while *E. coli* O157:H7 exceeded *Salmonella* in the sandy loam. Conversely, soil retention of *Salmonella* was significantly greater than *E. coli* in the silt loam soil but not different in the sandy loam. For *E. coli*, leaching losses and soil retention were significantly greater in undisturbed than tilled silt loam cores, but similar for *Salmonella*. Differential adhesion to soil aggregates and movement through natural soil water flow pathways likely contribute to behavior in the soil system of the two common enteric pathogens.

Kravchenko, Rose, and Smuker[72] have investigated the transport and survival of *E. coli* within soil aggregates. Soil aggregate samples were collected from three treatments: conventionally tilled, no-till, and 20 year-old native succession from a research site in Michigan. X-rayed microtomographic 3D images were obtained for 20 to 30 aggregates of each treatment. The work resulted in development of a methodology for conducting water flow, solute transport, and microbial transport experiments through the soil aggregates from coarse-textured soils with low aggregate stability. Unpublished results show substantial differences in pore structures of soil aggregates during two decades of contrasting management: those under native succession had overall lower porosity than those from conventional tillage agriculture, but the percent of large connected pores in native succession aggregates was larger than in the conventionally tilled soil aggregates. There was greater flux of chemical solutes and *E. coli* through the native succession aggregates, whereas *E. coli* retention was greater in the conventionally tilled aggregates. Thus, the ability of *E. coli* to be retained within the soil matrix differs not only among soil types, but also within the same soil subjected to different land use and management. The study also demonstrated the value of computer microtomography tools to help answer fate and transport questions. Additional research will address the fate of *E. coli* when the soil is subjected to saturated water flow during rainfall and runoff events.

Steenhuis, Hay, Richards, et al.[73] assessed farm-source *E. coli* and *Salmonella* mobility to water to understand pathogen fate and mobility in agricultural systems. Over the course of a month, bacterial sampling was conducted at three dairy farms in the Finger Lakes region of New York at various

---

[72] Kravchenko A, Rose J, Smucker A. Transport and survival of *Escherichia coli* within soil aggregates. Contract no.: 2008-35102-04567.

[73] Steenhuis TS, Hay AG, Richards BK, et al. Assessing farm-source *E. coli* and *Salmonella* mobility to water. Contract no.: 2008-35102-04462.

locations on the farm (cow housing, manure storage, field soil, drainage effluent) along the barn-to-field drain hydrologic flowpath. Isolates were then analyzed for phenotypic make-up and transport characteristics. A subset was further examined for subsurface mobility in sand columns. Preliminary results reveal certain trends. The subsurface soil and presence of drain tiles tend to select for a certain subset of *E. coli* strains, which the authors theorize may be better adapted for environmental survival and/or transport. Knowing which *E. coli* strains are more likely to be transported to drainage systems based on simple phenotypic characteristics could result in the development of better management strategies to control the spread of pathogens. Published results reported that the presence of tafi (a component of biofilms) did not retard the transport of *Salmonella*.[74] This finding stands in contrast to other studies with other bacteria that indicate that biofilms can act as a transport repressor. An additional value of the project was that it resulted in the development of an innovative method for sampling microorganisms from the artificial drainage effluent (using Moore swabs affixed to a pole, inserted into the drainage pipe).

The Steenhuis research group conducted basic research on bacterial transport in sand, examining effects of biofilm formation,[75] extracellular appendage production,[89] and also of two key constituents of dissolved organic matter, humic (HA) and fulvic acid (FA).[90] Investigation demonstrates that biofilm formation may limit downward transport in the near term, but can increase deep transport following heavy precipitation events. Visualizations have demonstrated the physical impedance to the flow field caused by the biofilm growth. Extracellular appendage production was found to generally cause higher retention of *E. coli* cells, but did not consistently affect *Salmonella* strains under transient infiltration events. In terms of DOM, sand-packed column experiments were conducted to understand how and under what chemical conditions DOM enhances colloid transport through unsaturated

---

[74] Salvucci AE, Zhang W, Morales VL, et al. The impact of biofilm-forming potential and tafi production on transport of environmental *Salmonella* through unsaturated porous media. *Biologia* 2009;3:460-464.

[75] Steenhuis TS, Hay AG, Richards BK, et al. Visualization and quantification of bacterial transport in sand under steady and transient flow. Contract no.: 2005-35102-16316.

[89] Salvucci AE, Zhang W, Morales VL, et al. The impact of biofilm-forming potential and tafi production on transport of environmental *Salmonella* through unsaturated porous media. *Biologia*. 2009;3:460-464.

[90] Morales VL, Zhang W, Gao B, et al. Impact of dissolved organic matter on colloid transport in the vadose zone: deterministic approximation of transport deposition coefficients from polymer coating characteristics. Submitted to *Water Res*.

porous medium. Experimental results indicate that HA improved colloid transport significantly by coating the colloids with a highly charged and thick brush layer, while FA only marginal affected transport despite having a large effect on particle charge. This research should help improve predictive models and therefore our ability to mitigate and prevent pathogen transport.

In eastern North Carolina, Noble[76] has worked to characterize the runoff microbial contaminant signature in two watersheds with differing land uses (septic systems versus agricultural lands), and is conducting fate and transport studies during a range of storm events. The primary goals of the study are to determine: 1) whether there is a contamination signature difference between creeks with contrasting land use, and 2) how much of the measured water column concentration during baseflow and stormflow is attributed to resuspended reservoir bacteria. During a wide range of storms and baseflow periods, water and sediment samples have been collected to quantify the fecal indicator bacteria (FIB) *E. coli* and *Enterococcus* and make simultaneous flow measurements. The molecular techniques of quantitative polymerase chain reaction (qPCR) and terminal fragment restriction length polymorphism, as well as conventional culture-based techniques, are being used to characterize the contamination profile in each creek. Land use adjacent to Ware Creek is equally row crop agriculture and single-family homes, whereas Oyster Creek is dominated by forest with nearby residential areas. Preliminary fecal coliform levels (as indicated by *E. coli*) in both creeks greatly exceed the regulated level during a high percentage of both dry weather and wet weather events. *Enterococcus* levels also continuously exceed the recreational water quality standard for North Carolina. Although FIB loading is significant at both creeks during rain events, loading at Ware Creek averages higher than Oyster Creek. *Bacteroides spp*., which are abundant in and indicative of human-derived fecal contamination, were present in Ware Creek during a 2.8 cm storm. *Bacteroides spp*., however, were not detected during the same storm at Oyster Creek nor during a dry weather sampling event (< 2.54 cm in the previous three days) at either creek.

Although these data are preliminary, results from the study will ultimately increase understanding of microbial fate and transport in watersheds with differing land uses, and the data generated could be used in the development of conceptual models to identify BMPs for watershed management. An impetus for the work was that approximately 64,240 acres of

---

[76] Noble RT. Understanding dynamics of microbial contaminant fate and transport in rural and agricultural lands. Contract no.: 2008-35102-04640.

shellfish beds are currently closed in North Carolina due to high fecal coliform numbers. Non-point source storm runoff is a dominant contributor of fecal contamination from agriculture, residential areas, and wildlife to coastal waters. Few studies, however, have differentiated runoff contamination fate and transport between agriculture and residential areas. Identification of fecal contamination sources and the role of reservoir bacterial populations to high priority shellfishing areas will assist in appropriate selection of management and mitigation strategies. Protocols are being developed by the researchers for use by water quality agencies to enhance decisions regarding human health effects stemming from non-point source runoff, contaminant source identification, and management and mitigation of these sources. Education and training of local water quality personnel has also been initiated.

Similarly, Lipp, Jenkins, Lowrance, et al.[77] examined transport of bacterial pathogens and indicator organisms at the watershed scale to understand the watershed-level relationships among land use, sewage effluent, and water quality, and pathogen dissemination and transmission. Detailed maps of land use throughout the watershed in the Satilla River Basin in Georgia were constructed, and preliminary patterns in the detection of both *Salmonella* and *Campylobacter* as they relate to land use have emerged. While the authors hypothesized that areas of high concentration of poultry houses would correspond to increased pathogen detection, the highest rates of detection for *Salmonella* were found at the first in-stream station downstream of the single wastewater treatment plant (WWTP) in the basin under study; while *Campylobacter* was most frequently detected from the direct influent to the WWTP and the station immediately upstream of the WWTP. Lowest levels were found in upstream stations with little to no poultry production. Corresponding with greater basin loads (i.e., more drainage area), higher levels of detection were also more common in downstream sites than upstream. Of the 78 strains of *Salmonella enterica* subsp. *enterica* isolated, 16 different serotypes were identified. *Salmonella* Montevideo was the predominant serotype, represented by 18 isolates (23%), followed by *S.* Braenderup (11 isolates [14%]) and *S.* Saintpaul (10 isolates [13%]). Of the samples positive for *Campylobacter* spp., *C. jejuni* was the most prevalent species detected (45%, 29/64) followed by *C. lari* (33%, 21/64). Serotype or species diversity was lowest in the upstream stations and increased at the downstream stations. In particular, *Campylobacter* spp. was detected more frequently from the downstream stations, especially immediately downstream of the WWTP, that treated both domestic and poultry

---

[77] Lipp EK, Jenkins MB, Lowrance R, et al. Watershed scale transport of *Salmonella*, *Campylobacter*, and indicator organisms in the Satilla River Basin. Contract no.: 2006-35102-17328.

processing waste. Publications are in preparation for this work. The results of this study should add to our growing knowledge of the microbial ecology of two pathogens (*Salmonella* and *Campylobacter*) of public health concern in watersheds in which agriculture is intense and expanding.

Boehm[78] has been studying the presence of pathogens in streams, and its relation to land use. The research has tested whether streams that drain primarily agricultural watersheds contain more waterborne fecal indicator bacteria (FIB), *Salmonella*, *E. coli* O157:H7, *Bacteroidales* host-specific fecal markers, and shiga-toxin genes than streams draining primarily urban or forested watersheds. The study has also looked at whether the distribution and diversity of *Salmonella* differs with salinity, human and livestock densities, rainfall, and other environmental parameters.

Fourteen streams and coastal inlets were sampled. Samples were examined for the presence of *Salmonella* and *E. coli* O157:H7. Additionally, *E. coli* were screened for *stx* (shiga-toxin encoding) genes. DNA extracted from water samples was also screened for *Bacteroidales* human-, ruminant-, and porcine-specific markers. Concentrations of FIB and *Salmonella* varied significantly among land use types, with higher average concentrations in urban watersheds than forested or agricultural watersheds. No significant differences in detection of the host-specific markers, *E. coli* O157:H7, or the *stx* genes were observed among land use types. Areal loading coefficients were calculated for each watershed for the bacterial indicators and pathogens. Preliminary results suggest that these loading coefficients correlate to agricultural land uses. Overall, there was a higher incidence of *Salmonella* detection when there was rain in the seven days prior to sampling and concentrations of FIB and *Salmonella* were negatively correlated with salinity. These results are preliminary and the work on this project is on-going.

Schneider, Walter, Buckley, et al. [79,80] evaluated the role of roadside ditch networks on the transport of pathogens from agricultural runoff to downstream drinking water supplies within three watersheds in west-central New York. Seven ditch sites were identified, including four adjacent to agricultural fields and three adjacent to non-agricultural and largely forested land parcels. Ditch

---

[78] Boehm, A. *Salmonella* and shiga-toxin encoding genes in coastal streams in Central California: relation to land use. Contract no.: 2007-35102-18139.

[79] Schneider RL, Walter MT, Buckley DH. Road ditch networks: rapid conduits for transporting pathogens and nutrients in agricultural runoff to drinking water supply systems. Contract no.: 2007-35102-18396.

[80] Falbo K, Schneider RL, Buckley D, et al. Submitted to *J Environ Qual* August 2010.

monitoring was initiated in the spring, immediately following manure spreading on the farms; sampling continued into fall storm runoff events and into the following spring for another manure-spreading cycle. Samples were analyzed within 12 hours of collection for total coliforms, viable *E. coli*, pH, conductivity, and total suspended sediments. Sediments of the ditch bottoms were also sampled for viable *E. coli*.

Total coliforms and *E. coli* were detected in the ditch waters at levels as high as 250,000 counts/100 ml, and continued to be found throughout the summer, fall, and early spring at diminishing levels. A similar spike of high levels of total coliforms and *E. coli* was again detected in the ditch waters immediately following manure spreading that second spring. The researchers concluded that manure spreading is a major source of the organisms in the ditch waters, and that very high levels continue to be present in the weeks to months following manure spreading. (The non-agricultural sites demonstrated significantly lower coliform counts, but high concentrations of *E. coli*; the researchers posit that possible sources could be from deer and other wildlife, septic leachates, or runoff from farm vehicles that traverse the adjacent roads.) A parallel study modeling water flow via road ditches indicates that ditches do route stormwater runoff more efficiently to the downstream lake, which supplies drinking water to approximately 100,000 people. The combination of the findings of these studies – the presence of viable coliforms in the ditches for months after manure spreading, and the hydrodynamic findings about the ditches themselves – demonstrate that viable coliforms and *E. coli* are efficiently transported by ditches to drinking water supplies.

An interesting anecdote to this study is that one of the participating farmers stopped providing specific information on manure spreading times over concerns that the coliform data might create public concerns about his farm. Studies of this nature may reveal such sensitivities, about which researchers will need to be aware and prepared to provide strong rationale and/or incentives to participants.

3.3 Virus transport

Theoretically – that is, in terms of filtration theory and DVLO theory – retention of virus should be greater than retention of bacteria or protozoa. Yet that is not what is observed. It is not well understood how surface structure of virus contributes to its retention and transport. When animal waste is introduced to agricultural soil, pathogen transport might coincide with in situ colloid mobilization. Viruses have been observed to be transported from less than a meter to more than a kilometer in the subsurface, depending on the nature of the substrata. Many factors are involved, including the nature of the virus (structure of the surface proteins), flow rate, presence of organic matter, degree of saturation, soil type, pH, salt concentration, and conductivity,

Interestingly, most of the transport grants in this report examined bacteria and protozoa. There are few reports in the literature dealing with the transport of manure-origin viruses. Almost nothing is known about the transport and release of animal viruses originating from manure. Some studies do discuss the transport of virus in soil. One found that epikarst is capable of filtration of viruses and their subsequent remobilization.[81] Another that looked at sewage sludge (biosolids) spiked with human adenovirus found that environmental contamination by the virus is unlikely because of strong soil adsorption.[82] But in general, studies of virus transport appear to be lacking. Given that approximately half of all waterborne disease outbreaks are traced to groundwater in the U.S. every year from contamination by wastewater or sewage,[83] further research on the transport properties of those viruses of importance cited in Table 2 is recommended.

3.4 Protozoa transport

Protozoan environmental stages are fairly buoyant in water when compared to parasitic helminth eggs, which has implications for their ability to transport in moving water. *Giardia* and *Cryptosporidium* tend to move with the effluent water, while helminths tend to collect with solids in sewage treatment plants. Protozoan stages will settle in calm water and can settle more quickly if they become enmeshed with or bound to background material in the receiving water. Oocysts and cysts can also adhere strongly to soil and vegetation and be transported on these particles in water. Predacious protozoa and invertebrates such as shellfish and rotifers can ingest protozoa such as *Giardia, Cryptosporidium*, and *T. gondii*, but the eventual the fate of these ingested pathogens is unknown. Mechanical transport throughout the environment by arthropods and birds is also possible.

---

[81] Flynn RM, Sinreich M. Characterisation of virus transport and attenuation in epikarst using short pulse and prolonged injection multi-tracer testing. *Water Res* 2010;44:1138-49.

[82] Horswell J, Hewitt J, Prosser J, et al. Mobility and survival of Salmonella Typhimurium and human adenovirus from spiked sewage sludge applied to soil columns. *J Appl Microbiol* 2010;108: 104-114.

[83] Svraka S, Duzier E, Vennema H. Etiological role of viruses in outbreaks of acute gastroenteritis in the Netherlands from 1994 through 2005. *Curr Microbiol* 2007;37:23-27.

McEvoy[84] has investigated sources of *Cryptosporidium* in a North Dakotan river. The focus was to determine the species/genotype of *Cryptosporidium* from the Red River, and also from cattle in the Red River Valley, and then to determine the genetic relatedness of isolates from these sources. Unpublished data confirm contribution of cattle (and wildlife) to water contamination. For example, the same *C. andersoni* genotype was found both in cattle at a ranch and in river water samples up to 28 miles downstream of the ranch. The cattle were clearly linked to surface water contamination. Studies such as this, which include both species and genotype information, can enhance our understanding of animals as a source of pathogen pollution in water. The *Cryptosporidium* present were host-adapted and not generally associated with human disease. *C. andersoni* was the only species found in cattle (although the number of positive samples was low), and no *C. parvum* was found in the cattle. *C. andersoni* has only rarely been found in humans. With that said, these cattle could be a source of other pathogens that do infect people. Data from this study also demonstrated a relatively high prevalence of *C. bovis* and the “deer-like genotype” in six- to eight-month-old beef calves compared to cows older than 2 years.[85] Overall, 43/212 (20.3%) animals were positive; only five of these positives were from cows. *C. bovis*, the deer-like genotype, and *C. andersoni* were identified in 9.4, 6.6, and 1.4% of animals sampled, respectively. *C. parvum* was not identified in any of the positive samples. *C. andersoni* was only detected in cows.

Kuhlenschmidt,[86] as part of grant work looking at the fate of *Cryptosporidium* in watersheds, examined the deposition of oocysts on natural organic matter surfaces.[87] Using a radial stagnation point flow system, the kinetics of *C. parvum* on quartz surfaces and silica surfaces coated with Suwannee River natural organic matter were examined. Microscopic evidence of oocysts trapped in shallow secondary minimum energy wells (a condition in which the microorganism and a surface are interacting in a weak and reversible fashion) showed that some were washed away by radial flow, but some were

---

[84] McEvoy JM. Sources of Cryptosporidium in a rural North Dakotan river. Contract no.: 2006-35102-17237.

[85] Feltus DC, Giddings CW, Khaitsa ML, et al. High prevalence of *Cryptosporidium bovis* and the deer-like genotype in calves compared to mature cows in beef cow-calf operations. *Vet Parasitol* 2008;151:191-195

[86] Kuhlenschmidt M. Control of *Cryptosporidium* and rotavirus contamination in agricultural watersheds. Contract no.: 2006-35102-17344.

[87] Liu Y, Janjaroen D, Kuhlenschmidt MS, et al. Deposition of *Cryptosporidium parvum* oocysts on natural organic matter surfaces: microscopic evidence for secondary minimum deposition in a radial stagnation point flow cell. *Langmuir* 2009;25:1594-605.

able to transfer more deeply and become irreversibly deposited at lower ionic strengths.

Ray, Harvey, and Chorover[88] examined the effect of dissolved organic carbon like anionic surfactant, sodium dodecyl sulfate (SDS) on the transport of formalin-inactivated *Cryptosporidium parvum* oocysts and oocyst-sized microspheres in temperate agricultural soil from Illinois. The findings indicate that agricultural practices that use reclaimed water containing surfactants can enhance pathogen transport and cause subsequent contamination of surface and ground waters during storm events or through agricultural activities. Thus, considerable caution must be exercised before sewage and municipal wastewater reuse.[89]

In another study, it was found that *Cryptosporidium* oocysts can be transported through preferential flow paths even in tropical agricultural soils rich in clay and iron oxide. It was also found that the volcanic ash soils can act as reservoirs for oocysts and oocyst-sized pathogens due to the highly reversible nature of attachment occurring in these soils.[90] Thus, overland flow during heavy precipitation events can transport surface-attached oocysts and contaminate water sources.

The molecular mechanism of viable *Cryptosporidium* oocyst interaction at the mineral surface (the hematite (α-Fe_2O_3)-water interface) was examined by ATR-FTIR spectroscopy. The results of the study provide the first spectroscopic evidence for an adhesion mechanism of oocysts to mineral surfaces that goes beyond DLVO interactions.[91,92] Further studies on the ternary system of oocysts-SDS-hematite indicate that the presence of SDS significantly diminishes the

---

[88] Ray C, Harvey RW, Chorover J. Influence of soluble organic matter on *Cryptosporidium parvum* oocyst mobility in variable charge soils. Contract no.: 2006-35102-17192.

[89] Mohanram A, Ray C , Harvey RW, et al. Effect of anionic surfactant SDS on the transport and attachment behaviors of Cryptosporidium parvum oocysts and oocyst-sized microspheres being advected through granular porous media. Proceedings at India 2010: 3rd International Perspective on Current & Future State of Water Resources & the Environment at Indian Institute of Technology (IIT), Madras, India, January 5-7, 2010.

[90] Mohanram A, Ray C, Harvey RW, et al. Comparison of transport and attachment behaviors of *Cryptosporidium parvum* oocysts and oocysts-sized microspheres being advected through three granular porous media. *Water Res* 2010. doi:10.1016/j.watres.2010.06.015.

[91] Gao X, Chorover J. In-situ monitoring of *Cryptosporidium parvum* oocyst surface adhesion using ATR-FTIR spectroscopy. *Colloids Surf B Biointerfaces* 2009;71:169-176

[92] Gao X, Metge DW, Ray C, et al. Surface complexation of carboxylate adheres *Cryptosporidium parvum* oocysts to the hematite/water interface. *Environ Sci Technol* 2009;43:7423-7429.

adsorption of oocyst surface onto hematite.[93] These observations suggest that surfactants may significantly impact the mobility of *C. parvum* oocysts in the environment.

3.5 Transport as a function of motility

Bacteria are not simply swept along by currents; they have the intrinsic ability to move themselves by use of their pili or flagellae. They can twitch, swarm, and swim, attach to soil particles, and swim upstream under low flow. Hill and Walker[94] have looked extensively at the motility characteristics of bacterial pathogens and how this influences their transport at relatively small scales through the groundwater and in irrigation pipes.

Packed bed columns and radial stagnation point flow systems were used to investigate *Salmonella enterica* deposition kinetics onto quartz surfaces and sand.[95] The presence of flagella increased the retention of bacteria in both systems, indicating that non-motile strains can be transported further with laminar flow than flagellated strains. Flagellar motion clearly plays a role in deposition and retention through pores and irregularities on quartz surfaces. Furthermore, one of the two flagellated strains studied gained flagellar motion at late-exponential phase, suggesting that flagellae are used to swim toward the quartz pores and surface irregularities, normally inaccessible to non-flagellated bacteria. As-yet unpublished data from the study also found that under laminar flow and in restrictive geometries, motile *E. coli* and *Salmonella spp*. arc out a parabola whose shape features are a function of shear and are influenced by bacterium velocity and shape. These data will allow for the generation of a model of bacterial transport for predicting where bacteria travel in porous media, with applications in groundwater and irrigation systems.

---

[93] Gao X, Chorover J. Adsorption of sodium dodecyl sulfate (SDS) at ZnSe and a-Fe2O3 surfaces: combining infrared spectroscopy and batch uptake studies. *J Colloid Interface Sci* 2010;348:167–176.

[94] Hill JE, Walker SL. Fate of motile bacterial pathogens in the subsurface: determining the extent and influence of motility and counter-current movement on cell. Contract no.: 2007-35102-18262.

[95] Haznedaroglu BZ, Zorlu O, Hill JE, et al. Identifying the role of flagella in the transport of motile and nonmotile *Salmonella enterica* serovars. *Environ Sci Technol* 2010;44:4184-4190.

3.6 Facilitated transport

Darnault and Jacobson[96] have been studying surfactant-facilitated transport of *C. parvum* in soil. Preliminary laboratory tests to investigate the transport of particles (nano-size quantum dot particles) in unsaturated sandy porous media showed that the presence of non-ionic surfactant Triton X-100 in water infiltrating the porous media enhanced the movement and transport of particles. The authors hope the research will impact agricultural and environmental professionals involved in the management of soil and water resources and the development of best management practices for the land application of animal waste and surfactants. In addition to protozoa, facilitated transport may be especially important for viruses because of their small size.

3.7 Pathogen transport in the context of food safety

This document is not a food safety document, and the workshop participants were, for the most part, not food safety experts. Yet we would like to point out that there is an obvious nexus between pathogens in water and pathogens in food. All crops must be watered, and often, this occurs through irrigation, and through other water-food nexuses such as runoff that affects seafood such as shellfish.

Much of the produce grown in the world today is dependent upon irrigation. This is especially true in the western U.S., where almost all crops are irrigated. Most of these waters are stored in man-made reservoirs and transported in man-made irrigation systems. The dynamics of transport and fate of pathogens in these synthetic systems are virtually unknown. These are very complex managed hydrologic systems, which have many potential inputs of both animal and human pathogens. The impact of irrigation return flows, sediment reservoirs, stormwater run-off, and channel design on transport need to be understood. Best management practices for ensuring water that is safe for irrigation of produce crops eaten raw is a distinct area of need, especially given that, contamination of irrigation waters is believed responsible for several outbreaks of foodborne illness in the U.S. and Mexico.

Gap: Data are needed on transport of pathogens in man-made irrigation systems.

---

[96] Darnault C, Jacobson A. Surfactant-facilitated transport of *Cryptosporidium parvum* in soil. Contract no.: 2008-35102-19159.

As a case in point, unpasteurized fresh apple cider has been related to outbreaks of cryptosporidiosis, with some of these outbreaks have been associated with cattle in the orchards and the lack of good manufacturing practices in the cider-pressing facilities. [97,98,99] [100] Apples that had fallen on the ground were considered the major source of cider contamination.[101] The Santín study referenced above, which involved major producers of unpasteurized apple cider in Canada, showed no correlation between harvest technique and the presence of *Cryptosporidium* oocysts in their cider. That study did find oocysts in the water that was used to clean apples and on processing equipment, suggesting that water was a potential source of contamination.

Cryptosporidium oocysts are known to occur widely in surface waters.[102,103,104] Apple orchards are subject to intensive pest-control and nutritional sprayings.[105] In some locations apple orchards require frequent irrigation,[106] and sprinkler irrigation was shown to be the best method for fruit growth.[107] Growers often use open surface waters such as ponds, lakes, and streams, which are potentially exposed to wildlife and livestock, to irrigate their apple orchards. Several studies considered irrigation waters a major route of

---

[97] Blackburn BG, Mazurek JM, Hlavsa M, et al. Cryptosporidiosis associated with ozonated apple cider. *Emerg Inf Dis* 2006;12:684-686.

[98] Garcia L, Henderson J, Fabri M, et al. Potential sources of microbial contamination in unpasteurized apple cider. *J Food Prot* 2006;69:137-144.

[99] Centers for Disease Control and Prevention. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider - Connecticut and New York, October 1996. *MMWR* 1997;46:4-8.

[100] Millard PS, Gensheimer KF, Addiss DG, et al. An outbreak of cryptosporidiosis from fresh-pressed apple cider. *JAMA* 1994;272:1592-1596.

[101] Robertson LJ, Johannessen GS, Gjerde BK, et al. Microbiological analysis of seed sprouts in Norway. *Int J Food Microbiol* 2002;75:119-126.

[102] Rose JB, Lisle JT, LeChevallier MW. Waterborne cryptosporidiosis: incidence, outbreaks, and treatment strategies. In Fayer R, ed. *Cryptosporidium* and cryptosporidiosis. Boca Raton: CRC Press, 1997; 93–109.

[103] LeChevallier MW, Norton WD, Lee RG. Occurrence of *Giardia* and *Cryptosporidium* spp. in surface water supplies. *Appl Environ Microbiol* 1991;57:2610-2616.

[104] LeChevallier MW, Norton WD, Lee RG. *Giardia* and *Cryptosporidium* spp. in filtered drinking water supplies. *Appl Environ Microbiol* 1991;57:2617-2621.

[105] Pfeiffer DG. Virginia, West Virginia, and Maryland Cooperative extension. 2010 Spray Bulletin for Commercial Tree Fruit Growers, February 9, 2010. Available at: http://pubs.ext.vt.edu/456/456-419/456-419.pdf.

[106] Santín M, Trout JM, Xiao L, et al. Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. *Vet Parasitol* 2004;122:103-117.

[107] Gur A, Dasberg S, Schkolnik I, et al. The influence of method and frequency of irrigation on soil aeration and some biochemical responses of apple trees. *Irrig Sci* 1979;1:125-134.

contamination for fresh produce.[108,109,110] Evidence of firm adherence of oocysts to spinach leaves and apples from experimentally contaminated water, and the finding apples remained infectious after four weeks postharvest storage support the hypothesis this pathogen could be transported to humans from fresh produce irrigated with contaminated water.[111,112]

3.8 Extreme weather events

Because extreme weather events can result in extreme amounts of runoff, outbreaks may follow such events. Most agricultural BMPs (whether for the control of pathogens or other pollutants) are usually designed for a maximum storm event of a particular magnitude, for example, a 25-year storm. Events greater than design limits can generate runoff that can overtop physical barriers (e.g., berms, diversions, waterways) or overwhelm treatment systems (e.g., filter strips, buffers, constructed wetlands). Extreme events can also overwhelm manure storage lagoons, leading to catastrophic direct discharge of manure and other wastes into waterways. All of these conditions can rapidly deliver pathogens from their source(s) to water bodies. Although such extreme events can dramatically increase pathogen loads to the environment, failure of water treatment or other barriers to human exposure are generally required for an outbreak to occur. Whether prolonged drought or flash flooding, extreme weather can dramatically alter the effectiveness of BMPs and the transportation of pathogens into receiving waters.

North Dakota's Red River experienced record flooding during the spring thaw of 2009, coinciding with the research period of McEvoy, Clark, and Khan,[113] who examined the sources, fate, and transport of *Cryptosporidium* in the watershed comprising the Red River and its tributaries, providing insight into the

---

[108] Chaidez, C., M. Soto, P. Gortares and K. Mena. 2005. Occurrence of Cryptosporidium and Giardia in irrigation water and its impact on the fresh produce industry. Int. J. Environ. Health Res.15:339-345.

[109] Robertson LJ, Johannessen GS, Gjerde BK, et al. Microbiological analysis of seed sprouts in Norway. *Int J Food Microbiol* 2002;75:119-126.

[110] Rzeżutka A, Nichols RA, Connelly L, et al. Cryptosporidium oocysts on fresh produce from areas of high livestock production in Poland. *Int J Food Microbiol* 2010. DOI:10.1016/j.ijfoodmicro.2010.01.027.

[111] Macarisin D, Bauchan G, Fayer R. *Spinacia oleracea* L. leaf stomata harboring Cryptosporidium parvum oocysts: a potential threat to food safety. *Appl Environ Microbiol* 2010;76:555-559.

[112] Macarisin D, Santín M, Bauchan G, et al. Infectivity of Cryptosporidium parvum oocysts after storage of experimentally contaminated apples. *J Food Prot* 2010 (in press).

[113] McEvoy JM, Clark ME, Khan E. Source, fate and transport of cryptosporidium in a rural Midwestern watershed. Contract no.: 2008-35102-19260.

source and human health importance of cryptosporidia found in surface water. The host species for a number of *Cryptosporidium* genotypes previously reported in surface water have been identified. Also, human pathogenic cryptosporidia, *C. parvum* and *C. ubiquitum*, were identified in both squirrels and voles. The researchers have evidence that host population diversity and density can impact *Cryptosporidium* prevalence. Samples collected during the flood put *Cryptosporidium* prevalence at 69%. Based on the high prevalence of the cattle-adapted *C. andersoni*, cattle were likely to be the primary source. This preliminary work also emphasizes that the impacts of extreme weather events cannot be understated. The potential for increased vulnerability due to climate change, particularly through changes in regional precipitation patterns and expected increases in extreme events, remains a concern.

3.9 Proliferation of antimicrobial resistance

An analysis of existing literature on antibiotic resistance facilitated by on-farm use of antimicrobials is not the intent of this project. The ability of resistant pathogens to mobilize within and off of farms, however, does deserve some attention in the context of pathogen transport. How big the problem is and how on-farm management practices contribute to it has not been comprehensively assessed, and we propose this as an area of continued research. Farm management practices are probably important in moderating the development of antibiotic resistance, and this would be of great value to consider.

Gap: The risk posed by resistant pathogens is not entirely understood. More information is needed to quantify not only the development of antibiotic resistance on farms, but also the amount environmental transmission of resistant bacteria from production facilities and pastures, and the extent to which different farm management practices mitigate this transport.

Soils are a harsh environment for enteric bacteria. Because environmental stressors promote genetic exchange as a survival mechanism, this may lead to an increase in gene transfer and rise of multidrug resistant bacteria. Several antibiotic resistance genes are co-located on plasmids or other mobile genetic elements with virulence factors. Factors that increase genetic transfer between organisms, therefore, may yield more virulent strains of bacterial pathogens that are resistant to human use medicines.[114]

---

[114] Holden MTG, Hauser H, Sanders M, et al. Rapid evolution of virulence and drug resistance in the emerging zoonotic pathogen *Streptococcus suis*. *PLoS ONE* 2009;4:e6072.

To determine mechanisms of extracellular DNA transport and transfer, Nguyen and Zilles[115] investigated the potential for spread of antimicrobial resistance genes near animal facilities. The work, which is using *Azotobacter vinelandii* as a study subject, is ongoing, but results indicate that a solution's ionic composition, such as calcium concentration and natural organic matter in groundwater, influences the amount of DNA that is adsorbed and protected from enzymatic degradation, but do not have a strong influence on natural transformation.[116] Results suggest that the presence of oxytetracycline in solution or in adsorbed form at environmentally-relevant concentrations does not have a strong influence on natural transformation of *A. vinelandii*. The work indicates that adsorbed DNA is fully bioavailable and could be an important reservoir for environmental gene transfer. The researchers offer that mathematical modeling of natural transformation may need to include only the amount of DNA adsorbed to soil surfaces, not its conformation or the concentration of dissolved natural organic matter.

Mackie, Chee-Sanford, Krapac, et al.[117] have also looked at transport of antibiotic resistance genes and residues. The researchers studied the occurrence of bacterial tetracycline resistance genes (Tc^r) in groundwater underlying two swine confinement operations. After applying PCR to genomic DNA extracted from groundwater samples, four *tet* genes (a type of Tc^r) were detected.[118] In general, genes were detected in greatest frequency at sites close to and downgradient of the manure lagoon, rather than those at greater distances or upgradients. Background detection frequencies of *tet* genes in soil were close to zero, but immediately after manure injection, it was possible to detect all *tet* genes for which surveys were conducted in most soil samples. Over time, the detection frequency of some *tet* genes returned to near-zero, while others persisted for months or longer than a year. (These soil data are not yet published.) The dissemination of genes appeared to be affected by geological factors, such as the presence of a sand layer or the dynamics of groundwater flow.

---

[115] Nguyen TH, Zilles JL. Investigating the spread of antimicrobial resistance near animal facilities: mechanisms of extracellular DNA transport and transfer. Contract no.: 2008-35102-19143.

[116] Lu N, Zilles JL, Nguyen TH. Adsorption of extracellular chromosomal DNA and its effects on natural transformation of *Azotobacter vinelandii*. *Appl Environ Microbiol* 2010;76:4179-84.

[117] Mackie RI, Chee-Sanford J, Krapac, IG, et al. Antibiotic resistance genes and residues in water and soil in close proximity to swine production facilities. Contract no.: 2005-35102-16426.

[118] Mackie RI, Koike S, Krapac I, et al. Tetracycline residues and tetracycline resistance genes in groundwater impacted by swine production facilities. *Anim Biotechnol* 2006;17:157-176.

In summary, antibiotic resistance gene pools in soils were impacted by the addition of manure, and the animal waste seeping from unlined lagoons at the studied facilities impacted the dissemination of resistance genes into underlying groundwater. Such findings could influence the siting of CAFOs and their manure/lagoon management. The extent of contamination from CAFOs would likely depend on the type and amount of antibiotics used, and the transport and flow of resistance genes between pools in the environment. Making generalizations is difficult, however, as the magnitude and extent of antibiotic resistance gene migration likely varies with local hydro-geological conditions. The results of the study, as pointed out by the researchers, also highlight the difficulty of establishing proper negative controls for environmental antibiotic resistance work, and point to the existence of a "native" antibiotic resistance gene pool among the environmental microbiota. However, the spatial and temporal patterns of antibiotic resistance genes at these three sites suggests that exposure to swine waste is an important factor in the spread of antibiotic resistance. The researchers offer that a "gene ecology" perspective, which takes into account the characteristic that genes may differ in their capacity to find new hosts via horizontal gene transfer, will be important for assessing the impact of agricultural activities on antibiotic resistance, and published additional research on macrolide, lincosamide, and streptogramin resistance that emphasizes this point.[119]

A valuable side note to this discussion pertains to the levels of antimicrobial resistance found at organic farms. Additional published research from this grant[120] demonstrated that the organic swine farm included in the study had not employed antimicrobials for approximately five years prior to the sampling. Despite this, tetracycline resistance genes were present in the feed and groundwater of that farm, although the implication of this and determination of where they came from is not clear.

Many antibiotics are soluble and are excreted from animals in large quantities. The environmental transmission of antibiotics themselves is therefore a potential concern. Presence of antibiotics in the environment may provide further opportunity for development or maintenance of antibiotic resistance genes. The role of this process in overall development of antibiotic resistant bacterial populations should be studied. Antibiotic transport in the

---

[119] Koike S, Aminov RI, Yannarell AC, et al. Molecular ecology of Macrolide-Lincosamide-Streptogramin B Methylases in waste lagoons and subsurface waters associated with swine production. *Microb Ecol* 2009;59:487-498.

[120] Jindal A, Kocherginskaya S, Mehboob A, et al. Antimicrobial use and resistance in swine waste treatment systems. *Appl Environ Microbiol* 2006;72:7813-7820.

environment has been studied and information is available on distributions and major processes that govern the fate of these compounds, but we still do not have a comprehensive understanding of these issues in complex environmental systems. Heterogeneity, temporal variability, and other environmental complexities are major challenges. The long-term effects of low concentrations of antimicrobials disseminated in environmental systems are not well known, nor are microbial responses to concentrations well below the minimum inhibitory concentration. General dissemination of antibiotic resistance genes is the primary concern, no matter where the mutation occurs.

3.10 Microbial source tracking

Increasing attention is being paid not only to the detection or fecal indicator organisms, but also to their source. Microbial source tracing (MST) is one approach to this question, based on the assumption that appropriate methods and indicator bacteria can be found and characterized as to their human or animal origin.[121] Molecular methods are commonly employed for this purpose. Most methods rely on fingerprinting of bacteria, such as through ribotyping or repetitive PCR. Nucleotide sequencing, microarrays, and metagenomics may all be useful tools. Non-molecular methods, such as antibiotic resistance analysis, are also used. A common method of source determination is to sample both a suspected source and adjacent watershed and compare the results, although this method only works in very small watersheds.

Development of tests for viral markers could also be a useful microbial source tracking tool. Animal virus markers for many species are not well studied. Freshwater mollusks may also be used to concentrate viruses, and in fact clean mollusks could be introduced into watersheds as sentinel organisms.

In terms of tracking protozoa of importance, recent molecular advances have enabled host associations to be inferred for *Cryptosporidium*, and (to a lesser extent) for *G. duodenalis* and microsporidia. There is very little information available for source tracking of *Blastocystis* sp. In fact, there is no reliable source tracking technique for any pathogen, protozoan or otherwise.

Gap: There is no reliable source tracking technique for any pathogen.

---

[121] Simpson JM, Santo Domingo JW, Reasoner DJ. Microbial source tracing: state of the science. *Environ Sci Technol* 2002;36:5279-5288.

Obropta, Miskewitz, and Phelps[122] used microbial source tracking as a tool to investigate the fate and transport of fecal contamination by characterizing conditions such as flow rate, temperature, and pH the Upper Salem River in New Jersey. Water samples were also collected for bacterial and nutrient analysis. The field measurements were then used to prepare models. A Soil and Water Assessment Tool model of the Upper Salem River Watershed is being developed for both flow characteristics and bacterial sources. Data were also collected to determine fate and transport parameters associated with bovine *Bacteroides*. The ultimate goal is to use the field sampling data to pinpoint sources of nutrient and bacterial contamination, and propose corrective measures. The model is expected to aid in this effort by contributing realistic estimates of the impact of dairy operations on bacterial contamination in small rural watersheds.

Dick, Moore, and Rajashekara[123] evaluated an Ohio watershed as a model for the study of pathogen origin, fate, and transport. The objectives of this study were to: 1) investigate correlations between land use patterns and microbial pathogen contamination, 2) identify the source of microbial pathogen contamination so that effective control strategies can be developed, and 3) determine the applicability of the MST method in the Upper Sugar Creek watershed. Culture-independent and library-independent, host-specific PCR and quantitative PCR assays (human and ruminant) were applied to identify the source of fecal contamination in the study watershed. The target sequence was the 16S ribosomal RNA gene of *Bacteroidales*, an indicator organism of pathogen contamination,

Initially, the effectiveness of sample processing protocols and long-term storage of environmental water samples was evaluated. Results indicated that membrane filtration for cell recovery, combined with the use of a small cell lysis container (2 mL size), produced the best efficiency and precision of DNA extraction. Long-term storage of environmental water samples before cell recovery and DNA extraction reduced DNA recovery rates.

The molecular-based, quantitative PCR assay was compared with the traditional viable *E. coli* count assay, and a significant ($p<0.001$) positive correlation was measured at low water flow rates. A high magnitude of general *Bacteroidales* qPCR signal was observed in samples from both a concentrated

---

[122] Obropta CO, Miskewitz RJ, Phelps C. Investigation of the fate and transport of fecal contamination using microbial source tracking. Contract no.: 2007-35102-18145.

[123] Dick WA, Moore RH, Rajashekara G. Upper Sugar Creek Watershed, Ohio: a model watershed for study of pathogen origin, fate and transport. Contract no.: 2007-35102-18217.

livestock operation area and a residential area. The ruminant- and human- host specific *Bacteroidales* PCR assays were also tested for their specificity and sensitivity with local fecal samples of potential hosts, and the result validated the use of these two host specific assays in the Upper Sugar Creek watershed. Frequent human-specific, positive *Bacteroidales* signals were also observed in water samples from a residential area. These results were followed up by a targeted sampling method at hotspots of microbial contamination within the watershed. The purpose of this targeted sampling was to accurately and cost-effectively identify the source of contamination. Spatially intensive samplings during baseflow water samples revealed that the most likely major source of fecal contamination in baseflow events was human origin from septic systems.

Temporally intensive samplings conducted during both surface water baseflow and stormflow conditions from suspected agricultural fecal contamination source areas indicated sediments could be a large potential reservoir of fecal contamination during stormflow events. Yet the fate and transport of two indicator bacteria, *Bacteroidales* and *E. coli,* indicated different transport behavior for these two organisms in storm flow versus baseflow conditions.

The MST method, when combined with targeted sampling, was able to identify sources of fecal contamination in a mixed-use watershed quickly, easily, accurately, and inexpensively. This research demonstrates the power of the MST method and also makes it an attractive tool in studies of pathogen contamination in stream water samples.

An additional study from the same grant looked at the effects of tilled versus no-till land, and rainfall on that land, on the transport of *C. parvum* oocysts.[124] Tilled and no-till soil blocks were treated with liquid dairy manure containing *C. parvum* oocysts. Even before any rain was applied, approximately 300 mL of water from the liquid manure – 30% of that applied – was transported through the no-till soil, but none moved through the tilled blocks. The blocks were then subjected to rainfall treatments and leachate was collected using a lysimeter at different time intervals. After rainfall, a greater number and percentage of first leachate samples from the no-till soil blocks compared to the tilled blocks tested positive for *Cryptosporidium* oocysts based on DNA extraction and amplification by PCR. Leachate broke through the no-till soil about 2.6 times as fast as it did the tilled soil, and greater numbers of oocysts were also recovered from the tilled soil than from the no-till soil using sucrose

---

[124] Ramirez NE, Wang P, Lejeune J, et al. Effect of tillage and rainfall on transport of manure-applied *Cryptosporidium parvum* oocysts through soil. *J Environ Qual* 2009;38:2394-2401.

flotation for identification. Although tillage was the most important factor affecting oocyst transport, rainfall timing and intensity also played a role. No-tillage has many agronomic and environmental benefits, but its practice can be improved. The authors suggest that methods of disrupting the direct linkage of surface soil to tile drains, which occurs via macropores in the soil created by earthworm activity, should be implemented to minimize transport of *Cryptosporidium* in no-till fields. As much as possible, manure should also be applied at least 48 hours prior to heavy rainfall.

3.11 Pores and drainage

Fox, Kanwar, Malone, et al.[125] looked at the role of biological macropores (a.k.a. "biopores") in *E. coli* transport through soil and into artificial subsurface drainage. Large portions of agricultural land in the Northeast and Midwest have this kind of drainage. Their work included both laboratory column studies with artificial macropores and *E. coli*, and tracer field experiments with rainfall simulation after liquid swine manure application.[126]

Previous research indicated that biopores and subsurface drains can be hydraulically connected, so the investigators sought to understand the importance of surface connected and disconnected (buried) biopores on *E. coli* transport when biopores are located near subsurface drains.[127] Field components of the experiments included infiltration tests, smoke tests to quantify directly connected macropores, liquid swine manure application, and monitoring of drain flows after artificial rainfall events. Biopore transport experiments were also performed. Laboratory experiments with artificial macropores demonstrated that in surface connected biopores, *E. coli* transport to subsurface drains was a function of the soil type and the layer thickness between the end of the biopore and drain. Buried biopores contributed flow and *E. coli* to the subsurface drain in less sorptive soils (i.e., loamy sand) and sorptive soils (i.e., sandy loam) containing a wide (i.e., with mesopores) pore space distribution prevalent due to the soil structure (i.e., packing technique). Sorption experiments are leading to improved multiple compound sorption equations for *E. coli* sorption to various soils after manure application.

---

[125] Fox G, Kanwar R, Malone R, et al. Role of directly connected macropores in pathogen transport to subsurface drainage. Contract no.: 2007-35102-18242.

[126] Fox GA, Kanwar R, Malone R. Earthworms and *E. coli*: a perilous combination for drain flow water quality. *Resource* 2008;15:22-24.

[127] Guzman J, Fox GA, Malone R, et al. *E. coli* transport from surface-applied manure to subsurface drains through artificial biopores. *J Environ Qual* 2009;38:2412-2421.

Field experiments are confirming the prominent role of directly connected macropores on transporting *E. coli* to subsurface drains, again indicating a larger number of directly connected macropores in no-till versus chisel-tilled plots. Fast breakthrough of bromide and *E. coli* through directly connected macropores was observed in biopore transport experiments. All of this work is leading toward the modification of a field-scale numerical model that includes artificial subsurface drainage (Root Zone Water Quality Model) capable of simulating macropore-facilitated *E. coli* transport.

Bradford, Walker, and Johnson[128] studied the role of pore structure, specifically as it relates to colloid-colloid interactions as a paradigm for pathogen transport. A variety of pore-scale studies were conducted to microscopically observe mechanisms of colloid and microorganism retention in porous media.[129] Batch and column scale studies elucidated mechanisms of colloid and microorganism retention in saturated and unsaturated systems. The influence of pore structure, solution chemistry (and transients), cell handling, water velocity, colloid concentration, surface macromolecules, and water saturation were investigated. Results indicate that colloid and microorganism retention in porous media is a highly coupled process sensitive to all of these factors, and the relative importance of each is highly dependent on the system conditions.

The research observations have demonstrated the importance of grain-grain contacts on colloid and microorganism retention under unfavorable conditions. Pore-scale simulations and observations have provided a mechanistic foundation to develop predictive theory for colloid retention in porous media. New continuum-scale modeling tools have been developed. Results are expected to aid in the design and development of efficient and cost-effective water treatment options for pathogens based upon soil passage. The development of improved models to predict the fate of pathogenic microorganisms in subsurface environments will ultimately facilitate the development of improved animal waste management practices. An improved understanding of microbe transport in the subsurface will also provide a

---

[128] Bradford SA, Walker SL, Johnson B. A new paradigm for pathogen transport and deposition: the role of pore structure and colloid-colloid interactions. Contract no.: 2006-35102-17388.

[129] For example: 1) Tong M, Ma H, Johnson WP. Funneling of flow into grain-to-grain contacts drives colloid-colloid aggregation in the presence of an energy barrier. *Environ Sci Technol* 2008;42:2826-2832. 2) Bradford SA, Kim HN, Haznedaroglu BZ, et al. Coupled factors influencing concentration dependent colloid transport and retention in saturated porous media. *Environ Sci Technol* 2009;43:6996-7002. 3) Kim HN, Bradford SA, Walker SL. *Escherichia coli* O157:H7 transport in saturated porous media: role of solution chemistry and surface macromolecules. *Environ Sci Technol* 2009;43:4340-4347. See Appendix for a complete list.

foundation for quantifying the fate and movement of other colloid-associated contaminants that may pose a threat to the public health.

In addition to their survival studies previously addressed, Jin, Sims, and Kniel[130] also performed column experiments to evaluate the transport and retention of ϕX174, MS2 and Aichi viruses in model porous media including metal oxide-coated sands in columns.[131] Goethite-coated and aluminum oxide-coated sands retained more viruses than oxide-removed sand. Aichi virus had slightly higher removal than ϕX174 and MS2. The adhesion forces between viruses and these different types of sands were measured by atomic force microscopy, possibly the first such effort to quantify virus-medium interactions directly. The authors state it is also the first study of the retention and transport behavior of the Aichi virus, a human enteric pathogen. These results enhance the body of understanding of virus retention, inactivation, and transport in porous media.

In another study, the researchers evaluated attachment potential of murine norovirus 1 (MNV) in biosolids, swine manure, and dairy manure to Romaine lettuce and internalization of this virus.[132] The MNV in animal manures had behavior similar to that of pure MNV; however, MNV in biosolids had significantly higher levels of attachment and internalization than pure MNV or MNV in manures. The incubation time did not affect the attachment of MNV in biosolids or manure. Confocal microscopy was used to observe MNV on lettuce after SYBR gold-labeled MNV was added directly to lettuce or after lettuce was submersed in labeled virus. MNV was observed on the lettuce surface, inside open cuts, and occasionally within stomata. In general, lettuce pieces with a long cut on the edge and short cuts on the stem was more likely to contain internalized MNV than intact lettuce pieces, as observed by confocal microscopy; however, while the difference was visible, it was not statistically significant. This study showed that the presence of MNV in biosolids may increase the risk of fresh produce contamination and that the MNV in open cuts and stomata is likely to be protected from sanitization.

---

[130] Jin Y, Sims JT, Kniel K. Effect of land application of wastes on the fate and transport of pathogens in soil. Contract no.: 2006-35102-17405.

[131] Attinti RJ, Wei K, Kniel JT, et al. Virus' (MS2, ΦX174 and Aichi) attachment on sand measured by atomic force microscopy and their transport through sand columns. *Environ Sci Technol* 2010, 44:2426-2432.

[132] Wei J, Jin Y, Sims JT, et al. Attachment and internalization of murine norovirus-1 in manure and biosolids to Romaine lettuce. *Appl Environ Microbiol* 2010;76:578-583.

Concluding Remarks and Key Points

Integration of the recent research on pathogen transport over multiple scales indicates that there is an overarching, unifying factor contributing to increased pathogen occurrence in water supplies, namely that the effects of increased human and livestock densities and the spreading of livestock manures and wastewater sludges need to be addressed.

While substantial progress has been made in identifying component processes, we still do not have the capability to predict how combinations of processes control overall migration of pathogens through watersheds. We can currently predict mean behavior fairly well, but not extreme release events or fate of low concentrations of infectious pathogens that are likely to produce outbreaks.

The work summarized in this chapter has elucidated many facets of pathogen transport, and the variables that affect it: transport-limiting and transport-enhancing effects of biofilms; retention capabilities of conventionally tilled soil aggregates; and the correlations between land use and pathogen loading and transport. Some the work has provided intriguing possibilities for transport related BMPs: for example, steel slag for reduction of *E. coli*; or occasional shallow-till after manure application to break up through-paths in long-term, no-till fields.

Despite these advances, transport processes are complex, and the various scales in which they are studied (e.g., association of single pathogens with environmental surfaces, and interactions in microbial communities and at the pore scale, column scale, field scale) remain fragmented. Many factors play a role, and many questions remain unanswered. Transport remains an excellent area for further study but, due to the cross-cutting nature of the work, there must be better collaboration between transport researchers and microbiologists at the very least, and, ideally, to include epidemiologists, producers, and others who may contribute relevant information and analysis. Key assessments made in this chapter include the following:

1. Progress has been made on understanding the physical and chemical mechanisms that govern pathogen retention on soils and sediments, but this knowledge has not been integrated into transport models that describe pathogen behavior at the field or watershed scale.

2. Our understanding of pathogen behavior is insufficient to thoroughly evaluate risks of pathogen transport and the spread of antimicrobial resistance.
3. Microbial source tracking research has made considerable progress, but effective tools that differentiate pathogens among farm animals and from humans and wildlife are not yet available.

4

Chapter 4: Sampling, Isolation, Identification, and Monitoring

Many variables go into the process of sampling – the what, the why, the how, the how many. Sampling strategy depends on the intent of the investigation. If one is interested in runoff, one would take samples from the surface environment. If groundwater penetration is the effect of interest, subsurface environments would instead be sampled. The instruments used, whether the sample has been preserved, and how soon it is analyzed all factor into how successfully an extraction and identification can be made. A major challenge in sampling is that the practice is limited by our ability to effectively concentrate samples such that we can detect organisms in the environment at levels relevant to their infectious dose.

The complexity of environmental media presents significant difficulties for pathogen detection and quantification work. Many methods that work well in the laboratory do not work well in environmental samples because of extraction difficulties. As a result, data on pathogen concentrations are fragmentary and may not be cross-comparable. In general, fecal indicator organisms do not provide a comprehensive picture of risks of pathogen transmission. "Indicators" are intended to do just that – give an indication of fecal contamination. Where found, the site should be followed up to examine more specific risks associated with particular pathogens. Given new measurement technologies, it is appropriate to consider if fecal indicators should be replaced or supplemented with direct measurement of a suite of organisms representing all of the major classes of pathogens. Research is needed to identify such a suitable suite of test organisms and methods.

Gap: Alternative quantitative methods to fecal coliforms as indicator organisms should be developed.

4.1 Sampling

Sampling for pathogens is often driven by assessment of drinking water, rather than to evaluate sources and environmental reservoirs of pathogens. Current methods for sampling pathogens are generally cumbersome and time consuming. This makes it difficult to obtain sufficient spatial and temporal coverage to track pathogen dynamics. Much more data are available on indicators, but, as discussed, it is not clear how well the indicator data reflects the transmission of various important pathogens.

No sampling method is without limitations. Culture and fluorescence microscopy-based methods are labor and time intensive, molecular methods are costly, genotype methods only allow detecting presence or absence. Every method has its specific applications of utility.

Gap: Sampling method limitations are an issue in terms of sensitivity, specificity, extraction efficiency, cost, limits of detection, and time for identification.

Gap: Sampling methods are cumbersome and time consuming. Improved technology for real-time in situ detection of indicators and/or specific pathogens would greatly improve our ability to understand linkages between pathogen sources, environmental reservoirs, and human infection.

Specialized sampling schemes are required based on local conditions, for example, tile drainage or ground water contamination. Sampling should focus especially on conditions that can lead to high loads, rapid export, and high concentrations of mobile pathogens, as these are the conditions likely to lead to high risk of disease transmission. Current sampling schemes generally do not capture transient behavior very well. Risk-based sampling schemes should reflect current understanding of hydrologic and transport dynamics. Sampling schemes should be developed to effectively assess all of the important reservoirs of pathogens; or alternatively, through risk assessment and hazard analysis identifying of critical control points, it may be possible to define sites of high risk that would serve as a sampling site under most scenarios.[133]

---

[133] Information on hazard analysis and risk assessment of critical control points (HACCP) can be found at the U.S. Food and Drug Administration's Web site: http://www.fda.gov/food/foodsafety/hazardanalysiscriticalcontrolpointshaccp/default.htm.

Known pathogens can be detected when they are present in sufficient quantities and tests are utilized correctly. And even when they are used correctly, the reliability of the result must be assessed by understanding the predictive value of the test, that is, that a positive test result is really indicative of a true positive. Predictive value requires knowledge of background prevalence, something which we do not often have. The information derived is also dependent on the methods used, and the sampling method chosen is dependent on the pathogen sought and the information sought.

The step of field sampling is critical. Where to take samples from and what sampling pattern and method to use will affect the results achieved. For any step in the process, design of appropriate sampling regimes is an objectives-driven process. In general, it is favorable to set automated sampling equipment to acquire flow-weighted samples rather than samples on timed intervals, so that load allocations can be determined from the data generated and bias toward base-flow conditions can be avoided.

Sampling methods can be grouped according to different matrices of interest, primarily feces, manure, soils, and water. Methods for FIB in surface waters are well established and standardized by the EPA. Many ongoing studies have sought to quantify the impact of hydrologic events on the loading of fecal indicators and pathogens to surface water bodies. These studies often employ automated sampling equipment for which methods regarding the sampling frequency and duration, sampling volumes, compositing, and even location of the intake mechanism have not been standardized.[134]

Standard methods are being developed by different entities for monitoring agricultural runoff. The USGS has provided a manual that includes chapters on collecting samples from ground water, surface water, and still water relative to biological indicators, such as fecal indicator bacteria, fecal indicator viruses, and protozoan pathogens.[135] The EPA also provides standard methods that apply to drinking water testing and monitoring for bacteria, viruses, and protozoa (see Appendix E). There is also another set of standard methods for

---

[134] In practice, the limits on hold times (fewer than eight hours before analysis) limit the effectiveness of automated equipment.

[135] United States Geological Survey. *National field manual for the collection of water-quality data: U.S. Geological Survey techniques of water-resources investigations, book 9, chaps. A1-A9.* Variously dated. Available at http://pubs.water.usgs.gov/twri9A.

the examination of water and wastewater.[136] Techniques employed include devices capable of acquiring flow-weighted discrete and/or composite samples (for example, automated sampling equipment or Coshocton wheel samplers). These types of samplers have been successfully deployed at the base of vegetative buffers, at outlets of tile drainage, within grassed waterways, and at transition points in waste management systems such as inlets and outlets of constructed wetlands (both subsurface and free water surface). Dustpan sample collectors have been used to acquire samples of surface runoff as it moves in sheet flow over buffers or at the edge of cropped fields. These samplers, however, are limited in that they provide a single grab sample that may not be entirely representative of overland discharge over the duration of any one event.

Additional laboratory studies would be needed to develop standard methods for pathogens. Most importantly, careful thought should be given to which pathogens should be sampled and what the ramifications for farmers would be, particularly if they were expected to sample their own farms. Alternative methods should be explored, for example, developing detection methods based on pathogen-specific metabolites. Any new standard validated methods for detecting pathogens should address cost, sensitivity, and specificity. Unfortunately, for all these methods, there is the problem that the more contaminated the water with particulates and pathogens, the harder it is to isolate and identify the pathogens of interest. Dispersed viruses and protozoa do not multiply in the environment, and thus, with dilution, are much harder to detect than when in a host.

Gap: Sampling methods are only standardized for *Salmonella*.

Gap: On-farm sampling methods are not overseen, and farmers are not necessarily provided with guidance on what method to use or how to apply it. The development of simple, farm-side tests may be of value both practically to the farmer and for epidemiological/research studies.

Gap: Improved hollow fiber ultrafilters are needed for enhanced virus recovery. In addition, virus recovery efficiencies should be established for these filters.

---

[136] Eaton AD, Clesceri LS, Rice EW, et al, eds. 2005. *Standard methods for the examination of water & wastewater: centennial 21st ed.* American Public Health Association; Water Environment Federation, American Water Works Association, 2005. Available at http://www.standardmethods.org/. (The methods are available at this site for a fee.)

4.2 Extraction challenges

One of the greatest challenges faced in determining if a pathogen is present is extracting the organism from the complex medium of wastewater, sludge, and/or manure in which it resides. Such extraction is particularly difficult for viruses and protozoa. Experts disagree about the best methods for extraction and analysis. With many approaches, sensitivity and detection are major issues. Furthermore, with so many different approaches being used, it becomes difficult to analyze separate data sets in an integrated way.

Interferences and sensitivity limits are significantly more problematic in soils, sediments, biofilms, and feces than they are in water samples. Extraction of pathogens and DNA from complex media is a major challenge. Interferences from complex organic matrixes and complex microbial communities lead to considerable uncertainty in distinguishing particular organisms. Concentrations of pathogens are often higher in feces, which makes sensitivity less of an issue for that source material.

Gap: The extraction step prior to molecular methods needs to be optimized.

Concentrating viruses from water (which only works for clean water samples) can be done by several means. One is hollow fiber ultrafiltration, in which cellular organisms are centrifuged out and the retentate is then examined for viruses. This procedure is relatively inexpensive and readily filters about ten liters of water. Charge-based filters do not perform well for some viruses, such as adenoviruses; set-up time, processing time, and field portability are also issues. New, once-through filters are becoming available, which will hopefully be relatively fast and field portable.

4.3 Detection and quantification

4.3.1 Bacteria

The only bacterial pathogen for which a standardized quantification method exists is that for *Salmonella*.[137] EPA Method 1682[138] (the "MPN

---

[137] Standardized methods also exist for fecal coliforms, which are not pathogenic.

[138] United States Environmental Protection Agency, 2006. Method 1682: Salmonella in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium. According to that report, "Method 1682 is a performance-based method for detecting *Salmonella* in biosolids.

method") is the standard method for detecting *Salmonella* in biosolids (sewage sludge) and has been generalized for research purposes for other matrices such as water. The method is currently being used for water, feces, soil, and sediment. This method that is designed specifically for the detection of *Salmonella* in sewage sludge is, in fact, the only standard method for quantifying bacterial pathogens in the United States.[139] Though the method is valid, it is imprecise; precision can be improved by increasing repetitions.

There is no standard method for other bacterial pathogens, although numerous methods similar to the MPN method are available, and in fact MPN may also be used on other bacterial pathogens besides *Salmonella*. A drawback of using the MPN method is that it may be limited by atypical phenotypes being overlooked, for instance, glucuronidase-negative *E. coli* strains.

Gap: Employment of different methods can confer huge variability, making comparisons between datasets difficult.

Gap: Current methods may miss pathogens that are present but below the detection limit of the method.

Quantitative PCR (qPCR) is a commonly used method for quantification of microorganisms. qPCR requires the identification of unique DNA sequences that can be amplified. It requires extraction of DNA from the sample which is subject to the problems discussed previously. Its sensitivity in terms of quantification is limited, but it presents good specificity, provided that the methodology is thoroughly tested. It does not allow for differentiation of live and dead organisms, for which new methods are being developed.

Method 1682 requires calculation of the MPN via enrichment, with selection and biochemical confirmation for determination of *Salmonella*. The enrichment step utilizes tryptic soy broth (TSB). After incubation, TSB is spotted onto selective modified semisolid Rappaport-Vassiliadis (MSRV) medium. Presumptively identified colonies are isolated on xylose-lysine desoxycholate agar (XLD). Biochemical confirmation includes lysine-iron agar (LIA), triple sugar iron agar (TSI), and urea broth, followed by serological typing using polyvalent O antisera. Calculations for concentration are based on dry weight." "MPN" stands for "most probable number."

[139] Note that standardized methods do exist for fecal coliforms: Method 1680: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using Lauryl Tryptose Broth (LTB) and EC Medium; and Method 1681: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using A-1 Medium.

4.3.2 Viruses

Methods for virus detection include conventional cell culture and various forms of PCR. Molecular detection methods that utilize PCR are rapidly pushing other viral detection methods aside. Although cell culture is the defined standard and can determine the infectious unit, it is also time consuming, offers non-specific identification, and is relatively expensive. Alternatively, PCR can detect non-cytopathogenic viruses, is specific, fast, highly sensitive, and relatively low in cost. The methods typically require a molecular sequence probe to get things started, but this is now becoming routine, and there is almost no longer a need for cell culture isolation or enhancement, nor serology to determine serotype, etc. The methods may utilize direct PCR or RT-PCR (reverse transcription PCR) for RNA viruses, and are becoming remarkably fast and sensitive. The real time PCR methodologies are allowing quantitative determinations of viral particles present in a given sample. Thus, if the sample can be extracted with a method that places the virus particle or genetic material into a tube small enough for PCR methods, the particles, and the apparent number of particles can be fairly routinely detected. Another advantage of these methods are that they also allow almost immediate sequencing, and therefore determination of species or subspecies of the isolated virus.

Gap: Inexpensive and rapid assays to confirm virus infectivity are needed.

4.3.3 Protozoa

There are no diagnostic methods designed for identification of the protozoan parasites of interest; they can only be concentrated within a given sample. For many of these parasites, samples from feces can be concentrated via flotation or sedimentation; from water, via filtration; and from soil, via sedimentation. Purification of oocyst or cysts from fecal samples can be achieved via density gradient centrifugation and immunomagnetic separation (IMS). For water or soil samples, there are no standard methods, except for *Giardia* and *Cryptosporidium*, for which IMS should be used when purifying cysts/oocysts from water samples. Microscopy, immunoassays, and molecular methods may be used for identification of many of the most important protozoa after they have been concentrated and purified from water. Most have no standardized method of identification from feces or soil but the same methods that are used for water are frequently employed with variable success.

Gap: There are no standardized methods for purification and identification of many protozoa from a variety of different media.

The standardized Method 1623 (filtration/IMS/FA) for identification of *G. duodenalis* and *Cryptosporidium* spp. in water is expensive and time intensive. Microscopy can be used to enumerate parasites and identify co-infections, but is skill-dependent and has low sensitivity. Immunofluorescence microscopy can offer genus-specific identification and is more sensitive than acid-fast staining. Acid-fast staining can be used to identify some pathogens, but can produce false positives with an unskilled technician, and is also non-specific.

Enzyme immunoassays are rapid, low-cost and easy to perform. However, these are only used for fecal samples, and are only genus-specific. They can, however, produce false positives, and are currently used mainly to monitor single samples from individual animals/people for the purpose of identifying a disease agent (e.g., clinical use).

Molecular methods have several advantages. They are highly specific and sensitive, can be used for feces, soil, and water samples, and can differentiate species and (sub)genotypes. The drawbacks with these methods are that they present high initial equipment and running costs, and require training to perform and specialized knowledge to interpret correctly. In addition, there can be cross-contamination problems, and the presence of inhibitors may reduce the sensitivity of the test. These inhibitor substances appear in variable and unpredictable levels in samples of different types (e.g., manure versus soil), leading to the potential for biases in extraction.

Gap: PCR inhibitors are a ubiquitous problem and can bias extraction/detection procedures, especially if appropriate and validated internal controls are not used. What the inhibitors are, and what effects they have on PCR, are incompletely known.

Molecular tools have been developed to detect and differentiate *Cryptosporidium* at the species/genotype and subtype levels. These tools have been increasingly used in characterizing the transmission of *Cryptosporidium* spp. in humans and animals. Small subunit rRNA based tools are generally used in genotyping *Cryptosporidium* in humans, animals, and water samples. This is largely due to the multi-copy nature of the gene and presence of semi-conserved and hyper-variable regions, making design of genus-specific primers easier. The *Cryptosporidium* oocyst wall protein (COWP) gene has also been used. In general, this and other genes only amplify DNA of *C. parvum, C. hominis, C. meleagridis,* and species/genotypes closely related to *C. parvum.* Human, animal, and environmental studies using these other genes tend to show fewer

Cryptosporidium species and genotypes than expected. COWP-based tools have limited usefulness in genotyping *Cryptosporidium* spp. of animals because of their narrow specificity. Subtyping tools are available to study transmission of *C. hominis* in humans and *C. parvum* in humans and ruminants. One common subtyping tool is DNA sequence analysis of the 60 kDa glycoprotein (gp60, also called gp40/15), which can categorize *C. parvum* and *C. hominis* each to several subtype families.

Similar methods have been developed for the identification of different isolates of *Giardia* from animal feces and environmental samples. It is with these molecular methods and the examination of the sequences of various genes (ssrRNA, Beta-Giardin, glutamate dehydrogenase, and the triosephosphate isomerase genes) that has allowed the discrimination of the *Giardia* assemblages and their relation to different animal hosts. Molecular methods are also being applied to *T. gondii*, *B. coli*, and *Blastocystis* sp.

4.4.4 Fungi

Molecular methods are being rapidly applied to various species of microsporidia. Santin and Fayer, using the internal transcribed spacer (ITS) region of the rRNA gene of *Enterocytozoon bieneusi*, have found that there are 81 genotypes with 111 genotype names: 26 genotypes have been identified exclusively in humans, eight have been identified in humans and in other hosts, 27 have been identified exclusively in cattle and pigs, six have been identified exclusively in cats and dogs, and 14 have been identified in miscellaneous hosts.[140] Looking at this agent in cattle using molecular methods from one week to 24 months of age, the overall prevalence was 24% (239/990) with a lower prevalence in calves less than 8 weeks of age and heifers than post-weaned calves.[141] Over 24 months, the cumulative prevalence of *E. bieneusi* was 100% since all 30 calves shed spores at some time. Several genotypes were found in the animals, but these genotypes all appeared to be cattle specific as they have not been found in humans or other animals. Similar molecular methods are also being applied to *Encephalitozoon cuniculi* and other species and genera within the group of fungal organisms.

---

[140] Santin M, Fayer R. *Enterocytozoon bieneusi* genotype nomenclature based on the internal transcribed spacer sequence: a consensus. *J Eukaryot Microbiol* 2009;56:34-38.

[141] Santin M, Fayer R. A longitudinal study of *Enterocytozoon bieneusi* in dairy cattle. *Parasitol Res* 2009; 105:141-144.

4.4 Identification and strain differentiation

The detection method employed is a critical step in planning, as it drives the collection, concentration, and storage method of choice. Options include cultivation, molecular methods, and direct counts (antibody, fluorescence in situ hybridization, microscopy, flow cytometry).

All bacterial pathogens that we would want to detect can be detected. Many standard cultivation-based methods already exist for bacterial pathogens (e.g., clinical, food safety, biosolids). Some standardized methods exist in other arenas that are applicable to manures and manure-amended soils. For instance, some EPA methods available for *Salmonella* in biosolids can be directly applied. Standardized methods for molecular-based approaches are, however, lacking. A multiple-technique approach may provide the best option for detection of pathogens in various environmental milieu. Many clinical methods for cultivation of bacterial pathogens do exist. The further removed from fecal samples, the less likely that these methods will work well in environmental samples considering variable matrix effects. New molecular detection methods offer great potential for high-throughput analysis, but are not currently reliable for quantitative analysis of diverse environmental samples.

Gap: Standardized methods for molecular-based approaches to pathogen identification are lacking.

PCR or RT-PCR methods detect DNA and RNA, respectively, even when the organism is not viable. PCR or RT-PCR may, therefore, not be optimal methods for applications in which the presence of live pathogens is of primary interest. Culture methods could be more appropriate for use in such cases. Pathogens from culturing methods can be used for subsequent genotyping and the possible linkage to outbreaks.

4.5 Sampling, isolation, and identification in the context of transport

Quantification is normally the focus in studies of environmental transmission. Molecular methods for differentiating strains of pathogens are available (e.g., rep-PCR, PFGE), but these are not routinely applied to transport data and watershed studies. These methods can be used to identify contamination sources, but trace-back methods and source tracking technologies are not routinely used at the watershed scale. Research is needed to verify utility of these tools. Source tracking could potentially be used with transport modeling to more explicitly link pathogen sources to infected individuals via particular environmental transmission pathways. There are major knowledge gaps in how to link sources to measured pathogen loads and to outbreaks. Improved conceptual models for linking microbial ecology and transmission are needed. Additional genotyping data would be very valuable to evaluate the relative importance of different transmission pathways.

Gap: Conceptual models to link pathogen source, genotype, transmission dynamics, and microbial ecology are lacking.

Transport experiments may benefit considerably from qPCR, which allows for relatively accurate quantification of the bacteria involved in the transport process. Both living and dead bacteria are accounted for in this method. Unfortunately, the accuracy of qPCR when applied to soil samples remains very low, once again due to the issue of adequate extraction. Nevertheless, qPCR tools should be explored for use in transport experiments. State-of-the-art tools used by microbiologists are often out of reach of transport researchers, since they require substantial microbiological expertise and expensive equipment.

Gap: Soil extraction methods should be optimized such that qPCR is a feasible means of study for transport experiments.

Participants stressed the importance of mass balance for transport modeling and for up-scaling microscale results to field/watershed scales. In order to mathematically model transport of pathogens, accurate information regarding the initial and final amounts of pathogens traveling through an area of interest (i.e., boundary conditions) must be known.

4.6 Monitoring for pathogen presence

Federal and state agencies rely on fecal indicators to predict the potential for illness from exposure to zoonotic pathogens in environmental waters. Using fecal indicators could over- or under-protect the public from actual pathogens. They do not completely correlate with pathogen transport and survival, and therefore pathogen presence. Coliform counts for groundwater and drinking water are not necessarily representative of pathogen loads. Research is underway to verify the efficacy of these indicators.

For now, however, fecal indicators are the best we have. The tests for them are simple, inexpensive, repeatable, and practical. Models to identify conditions and locations of vulnerability and quantify uncertainty in the use of fecal coliforms would be of considerable value, as would the development of indicators to more accurately predict risk.

Much as coliforms are used as indicators for bacterial pathogens, coliphages are viruses of bacteria that may be used for the same purpose. They also serve as a microbial source tracking tool. Studying the genetic makeup of coliphages may provide information regarding the source of fecal contamination. The focus should be on somatic coliphages (DNA viruses that infect host cells via the outer cell membrane).[142] They are diverse, persistent, and relatively abundant in animal and human feces, and the methods used to detect them are relatively inexpensive. PCR and probe methods are developed for these organisms. Somatic coliphages are found in different types of water, especially sewage. Microviridae are the most often found. They can be very useful tools as a marker for other viruses. For example, *Bacterioides fragilis* phages appear to work well as markers for human fecal contamination. The question of how do somatic coliphages compare to other viruses of interest in their response to environmental factors (UV, chlorine, pH, etc) is worthy of study. This information would be very useful for setting policy and making risk assessments.

Gap: Somatic coliphages as a tool for microbial source tracking should be investigated further.

Some level of ambient monitoring may allow for identification of unexpected drivers that lead to increased pathogen loading. Continuous monitoring may seem the obvious mechanism for true vigilance. Sensors (biosensors, nanosensors) might be helpful for continuous monitoring of the environment to identify critical points, although the cost and resource-intensiveness of this approach may make it unfeasible. Improved methods could make increased monitoring viable (cost, time, technical expertise, and could take the form of nanobiotechnologies. Current knowledge on transport processes can be very helpful in designing monitoring schemes to do the best possible job of capturing transmission.

---

[142] United States Environmental Protection Agency. *Method 1602: male-specific (f+) and somatic coliphage in water by single agar layer (sal) procedure*. EPA 821-R-01-029. Office of Water April 2001.

Concluding Remarks and Key Points

Sampling, isolation, identification, and monitoring are means to an end. They enable us to understand the threats we face, and where they come from. But they must be employed judiciously, not only because it is economically unfeasible to test all waters, but it is impractical and probably unnecessary.

Ultimately, all sampling, isolation, and identification science enables better monitoring. Drinking water is divided into two major types based on its source: surface water and groundwater (typically from wells). For these two sources, different treatment regulations apply. But even wells may require treatment, since groundwater can be impacted by contaminated surface water. Drinking water can be similarly categorized as from an individual or community system. The other major factor is whether people are drinking treated or untreated water. Most surface water supplies in the U.S. are treated water with processes in place for pathogen inactivation and removal. Groundwater, especially smaller wells, may be completely untreated, as it is not considered contaminated with surface water and surface pathogens. Thus, for the most part assessing specific pathogens entering the source water of a surface water drinking water plant might not provide large quantities of valuable information. At this time, it is difficult to foresee how ground water drinking water supplies could be routinely monitored for most manure-associated pathogens, except perhaps using certain agents as indicators of potential contamination.

Going forward, key initiatives should be to:

1. Standardized methods should be agreed upon and/or developed only for the detection of those water contaminant pathogens considered to be of particular importance;
2. Develop real time assay systems or field kits that yield results for indicators within two to six hours;
3. Develop indicators for viruses and protozoan parasites in water; and
4. Develop tools for microbial source tracking.

5

Chapter 5: Risk Assessment and Modeling

Why, despite the trillions of pathogens produced each day, are there so few outbreaks of waterborne disease? Is the risk lower than we might think? Determining what to do about pathogens in the watershed is fundamentally about understanding risk. What is the risk to the watershed (and to human and animal health) from pathogens in the watershed, and what source is the predominant contributor to this risk?

5.1 Determining risk

We can currently evaluate the overall risk of outbreaks, and the average environmental transmission behavior, but not the specific, local behaviors and processes that yield a high risk of outbreaks. Good progress has been made in identifying component processes, but we still lack the capability to predict extreme events, tails of distributions, and how combinations of rare events may interact to produce outbreaks. We thus cannot easily distinguish the factors that carry such extreme risk to justify harsh regulatory regimes, such as mandating certain BMPs or prohibiting certain agricultural or land use practices.

An understanding of pathogen fate and transport is critical for developing a predictive risk assessment model. Specific aspects of fate and transport vary by pathogen and by environmental conditions. Information capturing both spatial and temporal variability, including assessment of seasonal and life cycle patterns, as well as the ability to replicate extreme weather events, will be important for predictive risk assessment. Because of the large number of assumptions involved, however, the output predictions contain large uncertainties. For instance, fecal indicators, which are commonly used for risk assessment, may indicate a level of contamination, but may not provide sufficient information to quantify contaminants, nor be reflective of pathogen occurrence or numbers. Quantifying uncertainties is crucial, but regulators must understand that uncertainties will always exist. Better interactions between transport research and risk assessment research would be beneficial to addressing fate-transport components of the risk assessment models.

The conditions necessary for development of disease outbreaks are very specific, and factors contributing to these conditions are very dynamic. From a probabilistic perspective, a particular combination of levels of a large number of involved factors is required to produce an outbreak. With low probability of occurrence for a particular level of each factor, the cumulative probability of their occurring simultaneously becomes very low, thus resulting in a very low probability of disease outbreak.

With fate and transport models in particular, progress cannot be made without good data sets for validation models. Fecal indicator organisms may be useful for determining contamination, but there is still a need to monitor for pathogens when doing risk assessment.

Rare but extreme events cannot be ignored. Events such as storms and floods are required to mobilize large quantities of infectious pathogens, or to produce high concentrations of pathogens via resuspension and flushing. Rare events could also include exposure to small quantities of concentrated material, for instance, intense pulses, delivery of concentrated reservoirs (feces, sediments, amoebae infected with *Legionella*). In addition, extreme events may overload treatment plants, resulting in pathogens infiltrating the water delivery infrastructure. Improved predictive models would help to identify vulnerabilities arising from rare events, non-obvious interactions, multi-scale processes, and multiple barrier failures.

Gap: Improved predictive models are needed to identify vulnerabilities arising from rare events, non-obvious interactions, multi-scale processes, and multiple barrier failures.

What if we could predict indicator bacteria in real time? To determine the reliability and practicality of predicting FIB in real time, Haggard[143,144] looked

---

[143] Haggard BE. Fecal bacteria transport in Ozark streams. Contract no.: 2006-35102-17357.

[144] David MM, Haggard BE. Development of regression-based models to predict fecal bacteria numbers at select sites within the Illinois River Watershed, Arkansas and Oklahoma. *Water Air Soil Pollut* 2010. doi:10.1007/s11270-010-0497-7.

at the transport of FIB in Ozark streams. The researchers obtained bacteria and related chemical constituent data for the Illinois River (in Northeast Oklahoma and Northwest Arkansas) and select tributaries from the United States Geological Survey National Water Information Systems database. Using these data, they evaluated the relationship between FIB numbers and select physicochemical parameters (such as pH), utilizing regression models to predict daily FIB numbers. The prediction models were developed based on a synthetic approach considering two flow regimes: base flow conditions (BF) and storm runoff events (SRO).

Significant stepwise linear regression models were flow-, site-, and fecal bacteria-type specific. A greater number of significant regression models were developed during SRO than during BF. Turbidity and dissolved ion concentration were often correlated to fecal bacteria during SRO, a phenomenon that would benefit from further study. Findings emphasize the relevance of separating data into flow regimes. This separation was a useful step, providing a distinct difference in parameter relations and the reliability to predict fecal bacteria. The authors also emphasize the need to develop site-specific models to predict fecal indicator bacteria. The results indicated no clear trends for the streams within the basin as a whole; each stream and site location is unique and requires the development of individual, specific regression models to predict fecal bacteria numbers in real time. The authors propose that their approach may provide decision-makers with insight into real-time water quality conditions, and the types of models they developed may be utilized as planning tools to predict likely fecal bacteria numbers in streams.

Risk assessment may be one of the most challenging components of the study of pathogen transport, largely because much of the needed information for accurate assessments is still lacking. Identification and quantification of relevant pathogens must occur, transport mechanisms must be identified, and exposure probabilities and population demographics must be determined at each step along the entire pathway within the given watershed. Source tracking (and development of appropriate methods for this) is critical. Sentinel human populations that are most likely to be exposed must be identified and monitored. Social scientists must play a key role in evaluating risky behaviors that lead to transmission.

The basic steps in a quantitative microbial risk assessment are:

1. Identify the pathogen of concern;
2. Obtain dose-response data for humans;

3. Model infection probability;
4. Obtain clinical data to estimate probability of morbidity and mortality;
5. Predict the probability of disease from exposure; and
6. Validate model from outbreak data.

A variety of data sources may be used as inputs for these assessments. At the farm level, source occurrence in manure and incidence patterns in manure-producing animals (such as chickens, cattle, and swine) may be valuable. Environmental-level data, such as fate and transport information, climate and weather, and locations of outbreak occurrence, may be used as well. Human health components of a risk assessment could include morbidity and mortality levels, dose-response relationships, duration and magnitude of shedding, and the effects of immunity. Of course, knowledge of the zoonotic potential of a pathogen should inform a risk assessment, but as we have seen, this information is lacking in many cases.

The persistence of infective cells is a key driver of risk assessment. Additionally, rates for the various fate and transport processes are needed to make predictions of concentrations that are used as input into risk assessment models.

Exposure is also clearly a key linkage in zoonotic disease transmission. Exposure modeling is, therefore, critical for risk assessment. Exposure simulation requires having conceptual understanding and quantitative models for pathogen transport. This requires integration of small-scale processes over very large spatial scales and time scales, but no models are available for this upscaling. There is not even an accepted conceptual approach to this problem. The range of processes involved is enormous, so direct integration is not possible. Improved strategies are needed for multi-scale modeling approaches to identify transmission pathways from sources to receptors, and to evaluate pathogen delivery, deposition, resuspension, and survival in different environmental reservoirs.

The top priorities for performing risk analysis and its components are:

1. Clear formulation of the risk analysis, including input and buy-in from stakeholders;
2. Good exposure assessments driven by research, with clear identification of dynamic pathways and use of monitoring tools;
3. Good health effects data driven by research; and

4. Assessment of how specific interventions influence exposure.

Mohammed, Chang, McDonough, et al.[145] looked at ways to manage the risk from *Cryptosporidium* and *Salmonella spp*. in watersheds in the Upper Susquehanna Watershed in New York. The intent was to determine the prevalence of zoonotic genotypes of these pathogens in a random sample of dairy herds. Three risk groups of farms (low, moderate, and high risk) for each pathogen were identified. The investigators hypothesized that both endogenous (on-farm) and exogenous (from other sources) factors lead to the introduction, transmission, and perpetuation of these pathogens in the farm environment. Factors associated with the on-farm dynamics of these organisms were identified, and risk models were developed for each of them. The study also unraveled some of the ecological components of the watershed that affect transport pathways for *Cryptosporidium* and *Salmonella*. Intervention strategies on modifiable risk factors were considered via a decision tree analysis approach, which was used to evaluate their cost effectiveness.

Further discussion of risk analysis can be found in Chapter 7 under "Recommendations."

5.2 Modeling

Transport models may suggest optimal placement and location of barriers on a comprehensive "field to plate" route. If the probability of outbreaks is small, what is the best way to model these outbreaks? Traditional models are based on averages. The averaging approach does not apply to pathogen transport.

When modeling low probability outbreaks, stochastic modeling may be used. The focus should be on catastrophic/extreme weather events, especially relative to sensitive spots in the pathway. The risks associated with multiple barrier failures should be identified. Although extreme events matter most in disease outbreaks, modeling the occurrence of such events is not always practical due to the large number of factors involved and the low probabilities of occurrence of any one of these. Thus, modeling the averages and aiming at reducing the averages, thus shifting the population distributions to safer ranges will automatically reduce the probabilities of the dangerous extremes in the distribution tails.

---

[145] Mohammed HO, Chang YF, McDonough P, et al. Managing the risk of *Cryptosporidium* and *Salmonella* spp. in watersheds. Contract no.: 2006-35102-17356.

Modeling with accounting for all risk factors (temperature, precipitation, proximity to source) might be a way to handle risk assessment. Another would be to identify the situations with greater potential risk.

Classical models based on well-defined solution domains, highly simplified compositions, steady state, etc. are not appropriate. Direct integration or summation of isolated process descriptions is not likely to work because of mutually exclusive assumptions, spatial heterogeneity, non-linear process interactions, etc. Strictly correlation-based models are also not likely to work because of the high degree of complexity and variability in the system (non-stationarity, lack of data on extreme values, etc.) Risk assessment models need to consider both endemicity and rare events, and probably need to be stochastic in nature.

Probabilistic, predictive, process-based models are needed that include statistical distributions of most/all essential properties and processes. Extreme-value statistics will be needed to evaluate low-probability risks, and also general multi-variable probability distributions for processes that are not necessarily independent (e.g., distributions of cellular mobility and survival). New approaches are needed to identify dominant processes over a wide range of spatial and temporal scales: how processes at smaller scales affect larger-scale patterns, and how large-scale patterns constrain local behavior by establishing inputs, boundary conditions, etc. We also need to evaluate the extent to which any process found to be important at one scale affects behavior at larger scales (e.g., does bacterial motility significantly influence net pathogen migration at column or field scales?).

We are at an early stage in building predictive models for such large-scale environmental phenomena. Many groups are working to develop strategies for solving this type of problem. Predictions are not likely to ever be highly detailed (in the sense of providing exact descriptions of migration of small volumes of water or number of pathogens), but we should be able to develop models that include dominant controls and provide reasonable estimates of probability distributions for pathogen export, risk, etc. at specific locations and specific times (e.g., relative risk of having pathogens entering particular irrigation system or drinking water intake over time). This capability would be very useful for management. Models are required to evaluate the effect of proposed control measures, and to generate alternative scenarios for watershed management, development of regulations, etc. Models are also needed to forecast effects of land development, changes in water use and climate change.

Data on sources, agricultural practices, pathogen reservoirs, pathogen concentrations in transport, etc. are not widely available to explore multi-scale interactions and linkages. Watershed-scale databases are available for a variety of constituents (pathogens, nutrients, etc.) in many locations, but these data are not integrated or readily available for synthesis. Much less information is available on pathogens than on chemical constituents. Indicator data are more widely available, but are still limited. Increased use of well-instrumented watersheds with automatic sampling would be very useful for benchmarking different model approaches and to start to do risk assessments. (The USGS already maintains automated samplers on some watersheds.) In addition, one promising approach is to have real-time updating of forecasts based on ongoing measurements (as done with weather forecasting). Much more extensive, ongoing monitoring would be needed to support this type of forecasting systems for pathogen transmission through watersheds.

5.2.1 Scale of modeling processes

Uncertainty in bacterial transport modeling has presented challenges for determining bacterial fate and transport. To address this, Chen and Hilton[146] have modeled transport dynamics by way of column experiments, incorporating techniques that would help reduce uncertainty. The transport of *E. coli*, *Pseudomonas fluorescens*, and *Bacillus subtilis* in silica sand under water-unsaturated conditions was investigated.[147] Bacterial interactions were characterized based on bacterial and media surface thermodynamic properties determined by contact angle measurements. These calculated interactions provided solid evidence of the bacterial retention mechanisms in the pore system, helping to eliminate uncertainties arising with bacterial transport modeling. Based on this analysis of bacterial interactions, bacteria were found to be retained at the media-air-water three-phase interface, with the bacteria specifically attached to the air-water interface. Retention was calculated as the sum of the total "effective" forces exerted on the bacteria, that is, the attractive and repulsive interactions. Additional ongoing research is focused on the impact of physiological growth stage and macro nutrient ratio on *S.* Typhimurium and *E. coli* O157:H7 surface properties and transport in the subsurface soil. The researchers have thus far demonstrated that bacteria have different surface

---

[146] Chen G, Chan Hilton A. *S.* Typhimurium and *E. coli* 0157:H7 transport modeling for agricultural practices. Contract no.: 2007-35102-18111.

[147] Chen G. Bacterial interactions and transport in unsaturated porous media. *Colloids Surf B Biointerfaces* 2008;67:265-271.

properties when in different physiological stages and nutrient conditions. The work may ultimately provide strategies for animal waste land applications and reclaimed wastewater agricultural irrigation.

Gap: Different pathways require different models, with many challenges occurring at the interfaces of pathways. The need for integration of chemistry, biology, physics, and hydrology is an added challenge.

Concluding Remarks and Key Points

Predicting the occurrence of individual outbreaks might never be possible. Even though we lack detailed predictive, site-based models, the current knowledge base readily supports development general vulnerability maps for pathogen dissemination based on four to five well known factors that tend to lead to higher risk. Ultimately, we will probably be unable to go past probabilistic models, but we should be able to identify major controls on critical outcomes (transmission of large pulses of pathogens, delivery to food systems such as crops and aquaculture, and high likelihood of disease outbreaks). Prediction is currently limited by a lack of understanding of interaction of different processes (sources, transmission, survival, etc.) in complex environmental systems. Process-based exposure and risk models could provide a good framework for this type of prediction.

Grantees have made some significant progress in this area. Funded work has demonstrated, through modeling, that surface properties of bacteria differ under different physiological stages and nutrient conditions, which may ultimately lead to enhanced strategies for waste management. Modeling work is clearly a key component toward the ultimate goal of intervention. Research has also elucidated the need for individualized regression models to predict fecal indicator bacteria in real time.

Going forward:

1. Improved predictive models are needed, in particular to identify vulnerabilities arising from rare events, non-obvious interactions, multi-scale processes, and multiple barrier failures.
2. Interdisciplinary work in biology, chemistry, physics, and hydrology would help facilitate development of improved models.

6

Chapter 6: Remediation and Mitigation

What can we do to keep pathogens away from humans and animals? Controlling pathogens at their source is the ideal way to accomplish this, and the use of multiple barriers must be in place to achieve this end.

6.1 The concept of barriers

The concept of "barriers" is sufficiently broad to include a wide range of possibilities, from management practices to physical impediments. Examples include site selection, water and manure treatment, waste management (manure collection, storage, treatment, and land application practices), physical buffers, and grazing practices.

Current barrier approaches to transmission are fairly basic, such as reducing pathogen source loads, retaining material near sources with the use of flow and particle detention barriers (buffer strips, constructed wetlands), constructing barriers to water flow (diversions, berms) to protect certain areas, or encouraging die-off through storage, heating, composting, freezing, and thawing. A four-pronged approach for pathogen mitigation at the farm scale has been fairly consistently applied over the years: 1) keep it off the farm in the first place, 2) prevent cross-contamination of animals once on the farm, 3) manage manure appropriately, and 4) apply manure to land carefully. This approach works because, under most conditions, transmission of large quantities of infectious pathogens does not occur over long distances (i.e., away from the farm). Natural attenuation processes are generally severe – if they were not, pathogens would build up so extensively they would be much easier to find.[148]

Mitigation is nevertheless important. BMPs exist for the mitigation of non-point-source pollution caused by several water stressors of agricultural origin.[149] Vegetative and riparian buffers, wetlands, grassed waterways, terraced landscapes, and tile drainage have all been used effectively to reduce sediment and/or nutrient export from farm and crop environments. For example, a vegetated buffer can readily trap sediments due to reducing the velocity of surface water and infiltration into the soil profile. Nutrients such as phosphorous that can attach to sediment particles are likewise removed. That portion of surface flow that infiltrates into soil will function in most cases to substantially reduce pathogen loads due to subsurface filtration.

Similar co-benefits are seen with composting, a process commonly used by farmers with the intent of reducing pathogens, in addition to stabilizing manures to reduce odors and produce a better organic amendment for fertilization prior to land application. A well designed lagoon may also be perceived as a pathogen-reduction tool, even though many of those used on farms are not engineered for treatment and pathogen reduction as much as for meeting regulatory requirements for manure storage related to nutrient management planning and federal regulations.

Anaerobic digestion presents a similar case. This process has the benefit of some pathogen reduction, but is often not employed with that purpose. Although farmer responses to survey instruments indicate that they are interested in the pathogen reduction aspects of anaerobic digestion, they also indicate that the cost of the technology is just too high, and the process is thus typically implemented for the purpose of producing usable biogas.[150] An exception might be in dairy operations, where the dried organic solids or

---

[148] Satomi K, Jenkins M, Fogarty E, et al. *Cryptosporidium parvum* oocyst inactivation in field soil and its relation to soil characteristics: analyses using the geographic information systems. *Sci Total Environ* 2004;321:47-58.

[149] Those practices considered "best management practices" will vary. A useful collection of practices can be found in: the *Agricultural management practices catalogue for non-point source pollution prevention and water quality protection in New York State* (Table 1), the NRCS *National Handbook of Conservation Practices*, or those practices approved by the Watershed Agricultural Council as a component of an approved Whole Farm Plan.

[150] Gremelspacher M, Gillespie G, Welsh R. North Country New York dairy farmer views on alternative energy production. Unpublished report.

separated sand might be reused as bedding materials; this would reduce pathogens of interest, especially those that cause mastitis.

Some participants suggested, as an output of this paper, developing a list of BMPs for pathogen reduction. But pathogen-specific BMPs really do not exist; rather, there are other BMPs in use that also offer the benefit of pathogen reduction. Many of these are not well characterized or understood fundamentally, most likely because they lack a driver to exist (such as pathogen regulation). Regulators work under the assumption of pathogen control by nutrient and sediment BMPs, and nutrient-limited application.

BMPs that have co-benefits of pathogen reduction may also come at a cost. Despite their obvious value, there is strong evidence in the literature that some BMPs conflict between nutrient or sediment management and pathogen control, largely due to differences between nutrient and bacterial fate and transport pathways in agroecosystems. For instance, surface and subsurface drainage structures, such as tile drains and grassed waterways, are effective means of reducing sediment export from crop fields, but may result in increased loading of nutrients and/or pathogens to surface waters by short circuiting other BMPs (e.g., vegetative and riparian buffers) that would otherwise offer some degree of pathogen and nutrient control.[151,152,153] BMPs for manure treatment may similarly conflict between stressors. For instance, anaerobic digestion and composting can reduce concentrations of bacterial, viral, and protozoan pathogens by 2-4 log orders.[154,155,156,157] However, composting and anaerobic digestion may also reduce the agronomic value of manure by reducing nutrient

---

[151] Randall GW, Iragavarapu TK, Schmitt MA. Nutrient losses in subsurface drainage water from dairy manure and urea applied for corn. *J Environ Qual* 2000;29:1244–1252.

[152] Joy DM, Lee H, Reaume CM, et al. Microbial contamination of subsurface tile drainage water from field applications of liquid manure. *Can Agric Eng* 1998;40:153-160.

[153] Evans MR, Owens JD. Factors affecting the concentration of faecal bacteria in land drainage water. *J Gen Microbiol* 1972;71:477-485.

[154] Hutchinson ML, Walters LD, Avery SM, et al. Analyses of livestock production, waste storage, and pathogen levels and prevalences in farm manures. *Appl Environ Microbiol* 2005;71:1231-1236.

[155] Horan NJ, Fletcher L, Betmal SM, et al. Die-off of enteric bacterial pathogens during mesophilic anaerobic digestion. *Water Res* 2004;38:1113-1120.

[156] Kearney TE, Larkin MJ, Levett PN. The effect of slurry storage and anaerobic digestion on survival of pathogenic bacteria. *J Appl Bacteriol* 1993;74:86-93.

[157] Monteith HD, Shannon EE, Derbyshire JB. The inactivation of a bovine enterovirus and a bovine parovirus in cattle manure by anaerobic digestion, heat treatment, gamma irradiation, ensilage, and composting. *J Hyg Camb* 1986;97:175-184.

content and altering the form of nitrogen and phosphorus.[158] Anaerobic digestion also results in conversion of organic nitrogen to ammonia and increases the potential for subsequent production of nitrous oxide (a potent greenhouse gas). Lime stabilization may similarly reduce pathogens, but also increases ammonia release. Injection of raw or digested manures may break up soil macropores and reduce ammonia volatilization, but may also increase pathogen survival and mobility in manure-amended soils, as they are better protected from solar radiation and diurnal variability in temperature.[159] As a result, the watershed manager may choose to employ a specific BMP that targets the priority contaminant, be that sediment for a stream with endangered salmon, nutrients for a peri-urban lake that has become eutrophic, or pathogens for a rural watershed that functions as source of drinking water.

Gap: Producers lack practices for pathogen reduction from AFO point and non-point sources. A lack of understanding of basic biology and transport (as described earlier), and field testing and performance results, hampers development and refinement of practices.

How certain BMPs facilitate retention is still not well understood in all cases. For example, despite scientific evidence that free water surface and subsurface flow constructed wetland systems (CWS) can reduce pathogens from residential and agricultural waste effluents by 1- to 3-log orders depending on their specific configuration, very few studies discuss pathogen retention mechanisms *within* CWS.[160,161,162] In addition, data have been lacking on the CWS efficiency in pathogens reduction from diffuse agricultural pollution sources (runoff from AFOs, fields, etc.).

Gap: There is a lack of understanding of pathogen retention mechanisms as they relate to BMPs.

---

[158] This point is true especially regarding nitrogen loss through volatilization, but anecdotally, most workers in the field consider composting a useful technique to treat manure to reduce bulk, improve handling characteristics, and provide a consistent fertilizer. Composting is also a requirement in certain areas to allow manure to be applied to crops for human consumption.

[159] Hutchison ML, Walters LD, Moore A, et al. Effect of length of time before incorporation on survival of pathogenic bacteria present in livestock wastes applied to agricultural soil, *Appl Environ Microbiol* 2004;70:5111-5118.

[160] Chendorian M, Yates M, Villegas F. The fate and transport of viruses through surface water constructed wetlands. *J Environ Qual* 1998;27:1451-1458.

[161] Ferguson C, Husman AMD, Altavilla N, et al. Fate and transport of surface water pathogens in watersheds. *Crit Rev Environ Sci Technol* 2003;33:299-361.

[162] Sobsey MD, Khatib LA, Hill VR, et al. Pathogens in animal wastes and the impacts of waste management practices on their survival, transport, and fate. White paper for The National Center for Manure & Agricultural Waste Management, 2002. Available at: http://www.mwpshq.org.

Drizo and Gouli[163] studies at a Vermont dairy revealed a potential mechanism of *E. coli* reduction via filtration. Mechanisms of *E. coli* reduction in dairy effluent (mixed barnyard and milk parlor effluent) treated with innovative steel slag filtration technology (originally developed for phosphorus reduction) were investigated.[164]

In data being prepared for publication, numerous protozoa from the genus *Coleps* (Ciliatea, Gymnostomatida, Colepidae) were found on the steel slag surfaces, suggesting that this filtration material provide optimal microhabitat for these microbes, and as such may play an important role in regulating *E. coli*. Additional results revealed that steel slag filters have a very high *E. coli* removal efficiency. A single filter established at the University of Vermont dairy farm in Burlington, Vermont to treat mixed barnyard and milk parlor effluent, and having a volume of 3.7 m^3, operating at six days hydraulic residence time (HRT), and covering only 3 m^2 surface area had an average *E. coli* removal efficiency of 84.9%, and reduced *E. coli* from an average 3.03×10^6 per mL to 0.3×10^6 per mL (1 log) over a period of 300 days (August 2007 to May 2008) while two filters connected in series, having a volume of 7.4 m^3 achieved nearly 100% reduction (97.9%), from an average 3.03×10^6 per mL to 0.03×10^6 per mL (2 logs). Previous work on the effect of HRT on *E. coli* removal efficiency from a wetland pre-treated dairy effluent dairy farm revealed that *E. coli* removal efficiency was high at both 8 and 12 h HRT (75.7 and 81.75% respectively).[165]

Most BMPs for pathogen control are intended to retard water flow (increase residence time) and retain sediments. Removal normally depends on natural processes of retention and die-off. Performance for pathogen removal in BMPs is variable, often dependent on site-specific factors. Pathogens can be bound to particulate matter or planktonic in runoff water; pathogens are poorly removed by sedimentation. In the best of cases, pathogen removal may be similar to that of sediments in filter strips (90-99%).[166,167] However, considering

---

[163] Drizo A, Gouli V. Escherichia coli and Cryptosporidium occurrence, transport, fate and reduction from Vermont dairy farm point and non-point pollution sources. Contract no.: 2008-35102-19222.

[164] Drizo A, Gouli V, Twohig E. et al. *Escherichia coli* and *Cryptosporidium* occurrence, transport, fate and reduction from Vermont dairy farm point and non point pollution sources. Final grant report to the USDA National Institute of Food and Agriculture (NIFA), August 2010.

[165] Weber D, Drizo A, Twohig E, et al. Upgrading constructed wetlands phosphorus reduction from a dairy effluent using EAF steel slag filters. *Water Sci Technol* 2007;56:135–143.

[166] Dosskey, M.G. 2001. Toward Quantifying Water Pollution Abatement in Response to Installing Buffers on Crop Land. Environmental Management, 28(5), pp. 577–598.

high concentration loads to filter strips (often 10^4-10^6 *E. coli* per 100 mL) in manure-amended land, 1-2 log removal may be insufficiently protective of water quality in some cases.

6.2 Efficacy of BMPs on pathogen reduction

As discussed, a variety of BMPs exist to limit nutrient and sediment runoff from manure-amended lands. One of the questions asked by participants was, how well do these BMPs work? And in what ways do they fail? Current agricultural practices can actually work to increase the transport of manure pathogens from land-spread fields to downstream waters by directly connecting tile drains to roadside ditches.[168] Even after a single day of spreading, manure-based pathogens in this study continued to move through ditch networks for several months at concentrations well above conservative state and federal regulations.

To properly evaluate reductions achieved by individual practices and their treatment efficiencies, thorough monitoring using expensive automatic flow measuring equipment is a pre-requisite. As discussed earlier, this can be a major challenge for researchers given the lack of sources to financially support such investigations.

Gap: Additional research is needed on the effects that different management practices have on pathogen transport. The extremes are better elucidated than the intermediate practices and their resulting effects on pathogen transport.

Rogers and Shanks[169] have investigated fate and transport of pathogens originating in livestock manure applied to agricultural lands, and the performance of BMPs for limiting agricultural non-point source runoff during rainfall and snow-melt following manure application. In one study, nutrients, fecal indicator bacteria, bacterial pathogens, and antibiotic resistant bacteria were monitored in surface runoff (grassed waterway) and subsurface tile drainage of crop fields receiving swine waste lagoon effluent from a 5000-head

---

[167] For additional references, see Table 11, page 76 of: United States Environmental Protection Agency. *Detecting and mitigating the environmental impact of fecal pathogens originating from confined animal feeding operations: review*, by S. Rogers and J. Haines. EPA/600/R-06/021. US EPA National Risk Management Research Laboratory September 2005.

[168] Falbo K, Schneider RL, Buckley D, et al. Submitted to *J Environ Qual* August 2010.

[169] Rogers SW, Shanks O. Fate and transport of pathogenic microorganisms originating from livestock manures applied to agricultural lands. Contract no.: 2007-35102-18614.

confined swine finishing operation in the coastal plains region of North Carolina.[170] Both base flow conditions and rainfall-associated runoff of storms of varying intensity were investigated in drainage structures and upstream and downstream of the manure-amended fields. Bacterial pathogens and antibiotic-resistant bacteria were commonly detected in the receiving stream and in transition points in the treatment system. *E. coli*, enterococci and fecal Bacteroides measured in the stream adjacent to the manured field during storm flow exceeded populations measured during base flow by two to three orders of magnitude, suggesting insufficient residence time on the landscape to effectively inactivate pathogens. Bacterial transport from the manured fields occurred primarily in overland flow; as great as 11% of the load of fecal indicator bacteria to the stream adjacent the manured field was via discharge from a single grassed waterway. Although discharge of fecal indicator bacteria from tile drainage constituted less than 0.05% of the loading to the stream, drainage from a single tile accounted for as much as 71% of the nitrate load to the stream.

Management practices designed to impede movement of manure nutrients to surface waters may not be effective barriers for manure pathogens. Because of the conflicting nature of landscape management controls, effective management strategies should consider treatment practices that reduce pathogens prior to the application of manure to agricultural lands. As in the treatment of drinking water, multiple barrier approaches to protect human health, if affordable, are always indicated.

In another study by Rogers and Shanks, export of manure pollutants from crop fields with 100-foot setback distances following surface application of swine manure or turkey litter to frozen ground was explored. Nutrients, sediments, settleable and nonsettleable chemical oxygen demand, fecal indicator bacteria, bacterial pathogens, and antibiotic-resistant bacteria were monitored in snowmelt-associated runoff. Primary pollutant loads occurred in the first few runoff events, with manure-associated pollutants attenuating to concentrations similar to those measured from control plots receiving no manure within a month of application.[171]

---

[170] Liu D. Evaluation of Waterborne Pathogens Associated with a Concentrated Swine Feeding Operation in North Carolina. 2010, Clarkson University Center for the Environment: Potsdam. Masters of Science thesis.

[171] Owens LB, Bonta JV, Shipitalo MJ, et al. Effects of winter manure application in Ohio on the quality of surface runoff. *J Environ Qual* (accepted).

The Rogers and Shanks studies were designed to elucidate similarities and differences in the persistence of emerging fecal pollution indicators,[172] bacterial pathogens, and conventional cultivation-based measurements of fecal indicator bacteria in manure-amended soils, and in their transport properties in several BMPs. From field-based research, these investigators determined that emerging technologies offer improved resolution over conventional technologies for pathogen identification and source allocation, and also offer the advantage of greatly reduced analysis times. However, the sensitivity of the assays are reduced as compared to conventional cultivation-based methodologies. Through laboratory-scale studies, these researchers determined that genetic markers for *Enterococcus* spp., *E. coli*, and *Bacteroidales* exhibited similar decay rate coefficients as *E. coli* O157:H7, but not *Salmonella enterica* serovar Typhimurium, and persisted at detectable levels longer than both pathogens in manure-amended soils. Concentrations of the pathogens and qPCR genetic markers were correlated over the time course of their studies (r=0.528-0.745). Host-associated qPCR genetic markers decayed to non-detectable concentrations long before other fecal indicators, *S. enterica* serovar Typhimurium, and *E. coli* O157:H7. Although good indicators of point source or recent nonpoint source fecal contamination events, these host-associated qPCR genetic markers may not be reliable indicators of nonpoint source fecal contamination events that occur weeks following land application of manure.[173,174]

In addition to the analytical findings of the studies, another valuable result that may be of particular utility to other researchers has been the development of a physical collection of antibiotic-resistant *E. coli* and *Enterococcus spp*. bacteria originating from CAFO environments, as well as the production of a large data set on fecal indicator bacteria, bacterial pathogens, and host-specific PCR biomarkers in CAFO environments.

---

[172] As considered in this study, emerging fecal pollution indicators include real time quantitative PCR measurements of *E. coli*, *Enterococcus spp*., fecal *Bacteroidales*, and several manure pathogens, as well as several proposed (conventional and quantitative PCR) host-specific molecular biomarkers.

[173] Rogers SW, Donnelly M, Peed L, et al. Decay of bacterial pathogens, fecal indicators, and real-time quantitative PCR genetic markers in manure amended soils (in review).

[174] Donnelly M. 2009. Persistence of bacterial pathogens, fecal pollution indicators, and microbial source tracking markers in manure amended soils. 2009, Clarkson University Center for the Environment: Potsdam. Masters of Science thesis.

A study conducted by Albright[175,176] and continued by Jones[177] similarly sought to characterize a watershed and determine whether the influences of best management practices could be detected at the watershed scale. Albright monitored at 18 surface water and six groundwater sites for physical and chemical water quality parameters throughout the Brushy Creek watershed in southeastern Kentucky, and then used GIS to compare areas of the watershed with high adoption of cattle BMPs with areas of sparse or non-adoption. The results indicated that there were no differences in water quality at the watershed scale that could be attributed to the presence or absence of the BMPs, and hypothesized that one reason was that the karstic nature of the watershed was not considered in the development of the BMP prescriptions. A follow-on study in progress by Jones seeks to determine whether karstic groundwater-surface water interaction adequately explains why the cattle BMPs have had little effect on water quality by more completely characterizing the land- and hydrological conditions of the area, including land use characteristics, physical habitat assessment of riparian corridors, and censuses of fish and invertebrates. Preliminary results comparing areas of the watershed where there are groundwater-surface water interactions suggest that groundwater is, in fact, a substantial conduit of nutrient and *E. coli* contaminants in the stream network.

The problem of defining barriers that work for different stakeholders is manifest. The three major measures of watershed degradation by manure pollutants are phosphorus, nitrogen, and pathogens (as indicated by the presence of fecal coliform bacteria); it is these pollutants that barriers must target to minimize impact of manures on water quality degradation in the short term. Current BMPs are based on reduction of nitrogen and phosphorus loading; it would probably be worthwhile to determine to what extent these systems can be maximized to also minimize fecal coliform loading. *E. coli* is often the fecal indicator organism of choice to monitor in water quality studies. Therefore, barriers that minimize *E. coli* loading of waterways will do the most to reduce the perceived impact of farming systems on pathogens in waterways. This would have the greatest short-term positive effect on watershed impaction as determined by EPA methods, but may not necessarily be satisfactory to other

---

[175] Albright M. Assessment of agricultural best management practices in the brushy creek watershed. 2007, Eastern Kentucky University: Richmond. Masters of Science thesis.

[176] Albright M. Assessment of agricultural best management practices in the brushy creek watershed. Eastern *Kentucky Environmental Research Institute Report Summary Series*, 2007; RS07.005.

[177] Jones, A. Watershed scale assessment of a karst drainage basin using microbial, geospatial, and geochemical approaches. Contract no.: 2008-35102-19217.

stakeholders interested in the actual loading of overt pathogens (viruses, protozoan cysts, bacterial pathogens, etc.) from farm systems to surface waters. Instituting a voluntary tracking system for farmers to assess the benefits of their management practices on pathogen loading could prove quite useful. Again, we caution that wastewater treatment plants are not required to monitor their effluent for pathogens, and outright regulation of farmers to do so would be unfair.

After systems have been determined that minimize the loading of these three factors, additional work could focus on other organisms. Because the EPA already considers *Cryptosporidium* oocysts an agent of interest, it would be worthwhile, once the work on coliforms is complete, to add this organism to the list. The next major call of the AFRI-funded work is on noroviruses, so after oocysts it would seem likely that the fecal-sourced noroviruses could be the next target of mitigation. A logical process would be to build in an additional new target, only after BMPs have been maximized for the last group studied. The work would focus on improving the existing BMPs with additional modifications that would allow ever broadening dampers on watershed and waterway contamination with the pathogens of interest.

BMPs that prevent contact between “clean” runoff and manure/animal concentration areas, BMPs that promote infiltration (in some cases), and BMPs that control erosion may be the most important. Also, some older NRCS practices that manage manure (e.g., “waste utilization”) are beneficial to the extent that they set manure application back from streams, ditches, and other water courses. And even in the context of pathogens, nutrient management practices should not be discounted, as good nutrient management can reduce or prevent over-application of manure to agricultural lands, thereby reducing the availability of pathogens on the field.

What are the most effective suites of manure management practices for reducing pathogen load? Is it necessary to be so drastic as to recommend that there should be no manure application to produce-designated land?

Meals, Braun, and Hanzas[178] examined dairy manure management practices aimed at reducing pathogen runoff losses. The occurrence of microbial pathogens in representative dairy manure was quantified at three Vermont dairy farms. Data collected are being prepared for publication. Levels of generic *E. coli* in manure were consistent with values reported elsewhere, about 10^4 to 10^5

---

[178] Meals DW, Braun D, Hanzas JP. Assessment of dairy manure management practices to reduce pathogen runoff losses in agricultural watersheds. Contract no.: 2006-35102-17197.

MPN/g wet weight. No *E. coli* O157:H7 were ever detected, and only low numbers of *Giardia* and *Cryptosporidium* were observed in occasional samples. Because of the low number of pathogens detected in manure, *Salmonella* was added mid-study, and moderate levels were consistently detected in both fresh and stored manure. *E. coli* numbers were consistently at least an order of magnitude lower in stored manure compared to fresh manure. There appeared to be a seasonal pattern of lower *E. coli* counts in both fresh and stored manure during the late winter to early spring (approximately February through April). Mean *E. coli* levels in fresh manure declined by approximately 50% on all three farms during this period, then increased to previous levels for the remainder of the year. *E. coli* counts in stored manure followed a roughly similar pattern.

Samples from runoff events from paired corn and hay fields showed very low numbers of *Giardia*, *Cryptosporidium*, and *Salmonella*, with *E. coli* counts in the range of $10^2 – 10^6$ MPN/100 mL, depending on storm intensity and temporal proximity to manure application. Because of the low numbers of pathogens detected in either applied manure or field runoff, the effects of treatment were evaluated on the basis of generic *E. coli* data. Mean *E. coli* concentrations in runoff did not differ significantly between corn and hay land, but higher runoff volumes from corn fields drove significantly higher export of *E. coli* organisms compared to runoff from hay fields. Preliminary results from the paired-watershed analysis suggest that soil incorporation of manure on corn land did not have a significant effect on *E. coli* concentrations in field runoff, but did reduce export of *E. coli* organisms because incorporation significantly reduced runoff flows. Low vegetation height after hay cutting at the time of manure application appeared to lead to reduced *E. coli* counts in runoff and lower export of *E. coli* compared to the higher vegetation treatment. Evaluating the effects of timing of manure application with respect to rainfall events proved to be impossible due to farm management constraints.

Farm level BMPs, of course, are not the whole story. Regional dynamics are also very important in managing risks. Rainfall, temperature, probability of extreme events (flood/drought), drainage, erosion, and proximity to vulnerable populations or ecosystems must all be considered. Community groups with interest in water and its protection must work together to develop sustainable watershed management programs. In fact, drinking water facilities that supply treated surface water can get some small credit towards pathogen reduction if they work with communities to develop a watershed program. This is one means by which communities can earn disinfection credits with only a minimal input of capital. According to the EPA, "The watershed control program (WCP) credit provides the opportunity for public water systems with surface water sources employing filtration to obtain a 0.5-log credit from the Microbial Toolbox by developing and implementing a State-approved WCP plan. The elements of a State-approved WCP plan include identification of potential *Cryptosporidium* sources, prioritization of the identified sources, development of control measures to address the prioritized sources, and continuation of these efforts in the future. Systems with existing source water protection (SWP) efforts that meet these requirements can incorporate them into their State-approved WCP plan, while systems without existing programs can receive the same credit if they develop and implement similar SWP efforts as part of a WCP."[179]

6.3 Manure treatment

Manure has historically been applied to land to replenish nutrients and organics to soils where crops are raised. Thus, the materials on the farm are often handled with the goal being to land-apply the material for the purpose of cropland fertilization. Some farms separate liquids from solids for the purpose of composting or other "dry" disinfection or stabilization processes; the liquid portion, containing lower concentrations of BOD and fecal bacteria, is often land applied on fields with the assumption that there is continued biological degradation on the landscape.

Feces or manure are not always treated, disinfected, and/or stabilized prior to land application. In contrast, human waste residuals are often treated with the intent to land apply, and are thus similar to manures, except that the treatment standards for land application of human waste biosolids are more stringent than those for livestock manures.

Lagoons are a method of waste storage that has the collateral benefit of treatment and pathogen reduction. This may occur by aerobic or anaerobic processes. In anaerobic lagoons, unlike anaerobic digesters, there is typically no mixing of the material; it is simply pumped into the lagoon where the solids settle to the bottom. Further, most applications of these systems do not capture methane, ammonia, and other gases produced during anaerobic processes as do anaerobic digesters; however, caps can be installed on anaerobic lagoons for these purposes. Long residence times under anaerobic conditions in these lagoons and digesters favor the growth of acid-producing bacteria (acetogens and acidogens) as well as methanogenic archaea. As pathogens die off and

---

[179] United States Environmental Protection Agency. *Long term 2 enhanced surface water treatment rule: toolbox guidance manual*. EPA 815-D-09-001. Office of Water April 2010.

decay in this inhospitable environment, they become a food source for these and other organisms.

Often, the liquid fraction of an anaerobic lagoon is land applied within the confines of the required nutrient management plan that has been developed for the farm. Land application of the effluent might be on a regular basis, or may be only performed several times or during certain months of the year. The solids on the bottom of the lagoon may stay there for anywhere from months to many years. Typically, the solids are removed and land applied after the lagoon has filled to design capacity. In some cases, the solids are further treated before land application (e.g., dewatering, composting, etc.); in other situations they are land applied directly. Sometimes, the sludge might be taken to a landfill.

Crawford[180] has characterized the water quality of two treatment lagoons at the University of Idaho Dairy in Moscow, ID to study more feasible treatment schemes and pathogen reduction methods for dairy manure. Water samples were also analyzed from a nearby creek upstream and downstream from the dairy, as was lagooned manure from the dairy. Fecal coliforms were used as indicators of potential pathogens. Microscopic analyses indicated that the main organism in Lagoon I was a *Thiocapsa sp*., which was also present in Lagoon II (though to a much lesser extent). (*Thiocapsa* are nonmotile, Gram-negative, photosynthetic bacteria that use sulfur as an electron donor during photosynthesis.) The two lagoons differed by a 1000-fold coliform count, and their general bacterial diversity also greatly differed. To examine viral representation, primers were employed to test for the presence of well-studied phages. Evidence for enteric phages and their hosts was detected in both lagoons. A custom "phage-host" microarray was designed with thousands of probes for phages and their hosts, many of which were isolated from the lagoons, and a computer program was designed to analyze the results. This research is ongoing.

Additionally, Crawford hypothesized that dairy manure pathogens would be more effectively reduced by a novel, two-stage anaerobic digestion process that would transfer the solids from the fermenter to an anaerobic digester, and the volatile fatty acid-rich liquid to another treatment process. The research compared traditional processes to this novel process, by looking at the production rates of methane between the two systems, and the levels of pathogen reduction accomplished. The study is ongoing and the researchers are quantifying and confirming this pathogen reduction, as well as seeking to

---

[180] Crawford RL. Use of microarrays and QPCR/RT-QPCR for characterization of viral populations within water supplies affected by agricultural activities. Contract no.: 2008-35102-04635.

advance a biomarker. The work has tentatively confirmed that methane production is higher in the novel two stage system, as contrasted with a conventional single stage system.

Walker and Cwiertny[181] have studied photochemical disinfection of pathogens, in particular the effects of external polymeric substances (EPS) on reactive oxygen species (ROS). Their ultimate goal is to help develop cost-efficient treatment strategies that take advantage of natural attenuation of bacterial pathogens. One way to do this is via solar energy – photochemical processes that generate powerful ROS. The researchers hypothesized that the EPS layer that typically surrounds bacteria influences the rate of ROS-mediated disinfection. The level of EPS on the surface of *E. coli* was systematically modified for this purpose. Disinfection kinetics were analyzed, and preliminary observations have provided evidence counter to the original hypothesis. Specifically, in homogeneous systems with nitrate (which is responsible for hydroxyl radical production), the levels of EPS on *E. coli* appear to have no significant impact on the rate of cell die off. Nearly identical rates of cell die off have been observed regardless of the level of EPS removal prior to the photochemical disinfection test. The results indicate that indirect photolysis pathways involving hydroxyl radicals enhance rates of *E. coli* death when compared to direct photolysis alone. Additional control experiments showed that despite extended exposure to relatively high concentrations of singlet oxygen, bacterial viability remained high. The hydroxyl radicals had far more effect. The outcomes of this study will hopefully serve as a fundamental backbone of the development of the low-cost, low-tech, sunlight-mediated water treatment processes with the involvement of natural attenuation mechanisms and the impact of bacterial EPS. Fundamental insights will help farmers and regulatory agencies adequately manage water resources in the future, particularly with respect to the safe handling and reuse of agriculturally impacted waters. Additionally, results might also highlight future directions for engineered disinfection strategies that can harness and enhance photocatalytic mechanisms as a low-tech disinfection strategy, which has global implications for affordable forms of water treatment in developing countries.

In terms of protozoa, the parasites present in manure come in several forms. The pathogens of greatest interest for animal welfare are not necessarily the ones of most interest for watersheds because the majority of these parasites are not zoonotic agents. Thus, there is significant concern by the non-farming

---

[181] Walker SL, Cwiertny DM. Photochemical disinfection of pathogens: influence of extracellular polymeric substances on bactericidal capacity of reactive oxygen species. Contract no.: 2008-35102-04659.

community about only a small percentage of parasitic pathogens (mainly *C. parvum*); pathogens that, in fact, have not historically caused farmers many known, large production losses and which were long considered to be unimportant in the induction of human disease.

Bowman et al.[182] have studied mechanisms to minimize the impact of swine rearing facilities on their watersheds by abrogating or reducing the number of oocysts of *Cryptosporidium* species leaving the facilities in their waste streams. The oocysts of *Cryptosporidium* are more resistant to inactivation by swine waste lagoons treatment than *Salmonella*, enteric bacteria and viruses. Processes that effectively inactivate or minimize the transport of oocysts will have a greater capacity to inactivate, remove, or entrap viruses and bacteria present in swine waste. Because the oocysts of *Cryptosporidium* are likely to be present in most if not all swine lagoons, they may serve as indicators of the effectiveness of swine waste lagoons in pathogen reduction.

The work has examined the viability of oocysts present in lagoons, defined the effects of the lagooning of swine waste on oocyst inactivation, and evaluated the effects of pretreating swine waste in lagoons on the ability of oocysts to survive after land application. Ten swine waste lagoons associated with farrowing, nursery, finishing, and gestation operations were each sampled once a month for a year. Oocysts were extracted from triplicate 900 ml effluent samples, enumerated by microscopy, and assessed for viability by dye exclusion/vital stain assay. DNA was extracted from processed samples, and 18S rDNA genes amplified by PCR and sequenced for species and genotype identification. Oocysts were observed at each sampling time at each lagoon. Annual mean concentration of total oocysts and viable oocysts ranged between 24 and 51, and 0.6 and 12 oocysts ml^{-1} effluent, respectively. The distribution of species and genotypes was dominated (95% to 100%) by *C. suis*, and *Cryptosporidium* pig genotype II at eight of the lagoons. The lagoon at the gestation facility was dominated by *C. muris* (90%), and one farrowing facility showed a mix of pig genotypes, *C. muris*, and various genotypes of *C. parvum*. The zoonotic *C. parvum* bovine genotype was observed five times out of 407 18S

---

[182] Bowman DD, Jenkins M, Sharpe R. Survival kinetics of Cryptosporidium oocysts in swine facility wastes of the Southern Piedmont and coastal plain watersheds. Contract no.: 2006-35102-17191.

rDNA sequences analyzed. The results indicate that pigs can have mixed *Cryptosporidium* infections, but infection with *C. suis* is likely to be dominant.[183]

Work on the survival of oocysts, bacteria, and viruses in the lagoons is ongoing. Initial analyses have shown a marked die-off of oocysts in the lagoons in fairly short periods of time (i.e., months).

Drizo and Gouli[184] looked at *E. coli* and *Cryptosporidium* occurrence, transport, fate, and reduction from the University of Vermont dairy from point and non-point sources. Farm wastewater samples were collected monthly at four sampling points (barnyard settling pit, barnyard manure tank, dairy settling tank, and splitter tank/flume). Samples of feed bunk runoff generated during storm and snowmelt events were also collected from four sites. All samples contained *E. coli*, but with counts varying significantly by season. The authors suggest that *E. coli* is strongly affected by temperature fluctuations as well as farm practices that vary seasonally, such as quantity of germicidal chemicals used during winter and summer. Parasitic coccidians were not detected in any of the wastewater samples.

Another facet of this study was the inclusion of laboratory column experiments to investigate adsorption ability of steel slag. Columns filled with slag were used to investigate the effect of different hydraulic residence times (HRT) on *E. coli* reduction performance. In data being prepared for submission, *E. coli* reduction averaged 92% over nearly a year, which could provide significant benefit depending on the initial concentration. Column experiments confirmed that HRT affects the efficiency in *E. coli* reduction via steel slag filters, with increasing time leading to increased efficiency. Investigations on the ability of steel slag to reduce *Cryptosporidium* from wastewater using *Nosema* microspordia showed a decrease in the number of parasites. The investigators emphasize the importance of this finding, as it represents first demonstration of the ability of steel slag to reduce *Cryptosporidium* species from wastewater. This work, therefore, confirmed the efficacy of steel slag filters for reduction of *E. coli*, and, importantly, that steel slag can also be efficient in *Cryptosporidium* reduction. A collateral finding was the discovery of numerous *Coleps* protozoa on the surfaces of steel slag samples taken from the filter that had been treating

---

[183] Jenkins MB, Liotta JL, Lucio-Forster A, et al. Concentrations, viability, and distribution of *Cryptosporidium* genotypes in lagoons of swine facilities in Southern Piedmont and Coastal Plain watersheds of Georgia. *Appl Environ Microbiol* 2010;76:5757-5763.

[184] Drizo A, Gouli V. Escherichia coli and Cryptosporidium occurrence, transport, fate and reduction from Vermont dairy farm point and non-point pollution sources. Contract no.: 2008-35102-19222.

dairy effluent. Because these organisms feed on bacteria and other microorganisms, the authors suggest that they may play an important role in regulating *E. coli* concentrations in steel slag filters.

As with the other classes of pathogens, there are no virus-specific BMPs. Thermophilic processes, composting, ammonia, and other manure-associated natural antivirals, and high pH treatments all warrant further investigation. These processes all require increased cost for implementation. Thus, it is critical that risk assessment and risk analysis along with the costs of pathogen-reduction BMPs be considered relative to manure handling, because unlike drinking and wastewater treatment, the costs are borne by the farmer, not by a municipality. Increased BMP costs figure directly into increased commodity costs that will then be passed on to the consumer.

6.4 Physical buffers

The strategic solution to impeding pathogens from transporting to the watershed is the application of multiple barriers, reducing the overall average risk. There are potential inexpensive and effective physical buffers that could be used to reduce runoff and subsequent contamination. Vegetative buffers, for example, decrease surface flow rate, prevent runoff from directly entering surface water, and can retain some pathogens in the soil. Buffers include:

- Vegetative and riparian buffer strips;
- constructed wetlands; and
- fecal containment and processing.

Buffers and barriers can have other beneficial effects, as well. Riparian buffers reduce manure application near watercourses and help prevent accidental direct application of manure into waterways. Fences and hunting practices can limit wildlife access to farms. Fans, screens, and traps can prevent infiltration by birds and insects. All types of animal control can be viewed as buffers.

Atwill, Tate, and Yates[185] examined the efficacy of grassland buffers in containing pathogens to reduce waterborne concentrations of *Salmonella*, *C. parvum*, and rotavirus, three enteric pathogens of importance found in cattle. Unpublished data from the study demonstrated that buffers can substantially

---

[185] Atwill ER, Tate KW, Yates MV. Efficacy of grassland buffers for reducing *Salmonella*, *Cryptosporidium parvum*, and rotavirus in rangeland runoff. Contract no.: 2006-35102-17193.

reduce the number of waterborne *C. parvum* and *Salmonella* in rangeland runoff, when appropriately designed and managed. The study determined that as an intervention strategy, grassland buffers could be one of the more cost-effective methods for reducing pathogen discharge from animal agriculture, at least as it relates to the pathogens studied. Published extension work on *E. coli* and *C. parvum* revealed variables that influence the effectiveness of buffers. [186] For instance, as runoff volume increased, buffer efficiency decreased. There was a significant interaction between buffer width and total runoff per plot of land for *E. coli*, indicating that the effect of buffer width on discharge was in part dependent upon total runoff. Increasing slope also had a positive correlation to increased runoff.

Kuhlenschmidt[187] evaluated means of control of *Cryptosporidium* and rotavirus in agricultural watersheds by characterizing critical environmental factors affecting transport and control in runoff. The investigators analyzed the effect of soil conditions such as presence and type of vegetation on fate and transport. Results being prepared for publication suggest that soil is somewhat thermally protective for rotavirus: approximately 50% of virus infectivity could be recovered from intact soil following at least 18 days of incubation at 25°C, compared to only 20% for aqueous solution. In contrast to intact soil, sand appears able to either inactivate rotavirus or to bind it in a manner that inhibits its recovery following water extraction. *Cryptosporidium*, in contrast to rotavirus, appears to survive well under all environmental conditions tested, with infectivity declining only after two months of collection or deposition from the cow.

The study showed that overland transport of microbial pathogens was reduced by vegetation, by allowing for pathogen penetration into the soil profile and resultant adhesion to clay or sand particles. While the type of vegetation used was of minimal impact, the density of the vegetation and the type of soil had marked effects on the degree of pathogen retention. High-density vegetation in a high-sand content soil was the most effective combination.

---

[186] Tate KW, Atwill ER, Nader G, et al. Recent annual rangeland buffer and RDM study results. Proceedings for the Beef and Range Field Day: Sierra Foothill Research and Extension Center, University of California. April 19, 2007:21-24.

[187] Kuhlenschmidt M. Control of *Cryptosporidium* and rotavirus contamination in agricultural watersheds. Contract no.: 2006-35102-17344.

6.5 Grazing management

An evident area of interest has been grazing management. Russell, Ensley, and Yoon[188] investigated the effects of management practices in the Rathbun Lake watershed in southern Iowa and the Willow Creek watershed in central Iowa. Stream samples were collected at upstream and downstream sites from streams in 13 beef cattle pastures in the Rathbun Lake watershed. Fecal samples from cows and runoff samples from rainfall simulations on vegetated and bare sites on stream banks were collected in the Willow Creek watershed. Incidences of bovine enterovirus (BEV), bovine coronavirus (BCV), bovine rotavirus (BRV), and *E. coli* O157:H7 were measured in all fecal and water samples. Coliform counts were also determined in all water samples. In addition, because the risk of pathogen loading of pasture streams may be affected by the temporal-spatial distribution of grazing cattle, GPS was used to account for such variability.

Results published in an extension article[189] indicate that incidences of BEV in up- and downstream samples were related to the presence of cattle in the pasture for up to three days prior to sampling. However, there were also: high concentrations of coliform bacteria in the water of streams entering pastures; a lack of a net increase or an effect of stocking rate on the concentrations of coliforms in streams leaving pastures; and a lack of net increases in the incidences of BEV, BCV, and BRV between up- and downstream water samples. The researchers have therefore assessed that sources of coliforms and pathogenic viruses other than those from grazing cattle are major contributors to pathogen loading of pasture streams. The study also found little relation between the presence of coliforms and pathogenic viruses in pasture streams.

The study showed low frequency and seasonality of shedding of *E. coli* O157:H7 and the viral pathogens. The increased incidence of *E. coli* O157:H7 and BEV seen in the feces of calving cows in September suggests that autumn shedding may be aggravated by calving and that grazing practices should possibly take this risk into account. However, neither *E. coli* O157:H7 nor viral pathogens were ever found in streambank runoff samples, implying that grazing

---

[188] Russell JR, Ensley SM, Yoon KJ. Grazing management effects on pathogen loading of Midwestern pasture streams. Contract no.: 2007-35102-18115.

[189] Bear DA, Cho Y-I, Russell JR, et al. Incidence of bovine enterovirus, coronavirus, and group a rotavirus, and concentration of total coliforms in Midwestern pasture streams. Iowa State University 2010 Animal Industry Report. R2532.

cattle pose a small risk of pathogen loading of streams. Only BEV, a potential bovine fecal marker, was ever found in the runoff samples, at rates of 3.1% and 1.0% of runoff samples from unvegetated and vegetated sites, respectively, in pastures where cattle had unrestricted access to the streams.

So how can grazing practices minimize risk? Russell and colleagues posit that risk may be minimized by practices that reduce the proportion of bare ground or manure near pasture streams, such as restricting stream access to stabilized crossings or rotational stocking with flash grazing of riparian paddocks. In this study, restricting access of pasture streams by using stabilized crossings or rotational stocking reduced the percentages of time that cattle were in streams by 80% to 95%, and near (within 33 meters) of the streams by 70% to 81%. The authors note that the effectiveness of grazing management practices to reduce pathogen loading of pasture streams will depend on site characteristics such as area, shape, and shade distribution, and management practices must be targeted as such.

6.6 Site selection

The reality is that many variables – the slope of the land, regional climate, hydrologic properties, the presence of ponds – can affect pathogen transport from farms. It is outside the realm of feasibility to consider all of them when selecting a site for an agricultural operation. But understanding the variables and making informed decisions is nevertheless a legitimate strategy in the development and implementation of site-specific management to prevent future problems.

Jenkins[190] took an ecological approach in examining the role of ponds in reducing the threat of pathogen contamination. *Salmonella* and *E. coli* O157:H7 concentrations and die-off rates were compared to concentrations and die-off rates of FIB. This was achieved via the development of a culture-based MPN methodology for quantifying dilute concentrations and fluxes of *Salmonella* and *E. coli* O157:H7 in surface waters.[191,192] Use of this methodology provides a quantitative tool to assess the human health risk for surface water

---

[190] Jenkins MB. The role of ponds in reducing the threat of pathogen contamination from livestock in agricultural watersheds. Contract no.: 2005-35102-16374.

[191] Jenkins MB, Endale DM, Fisher DS. Most probable number methodology for quantifying dilute concentrations and fluxes of *Salmonella* in surface waters. *J Appl Microbiol* 2008;104:1562-1568.

[192] Jenkins MB, Endale DM, Fisher DS, et al. Most probable number methodology for quantifying dilute concentrations and fluxes of *Escherichia coli* O157:H7 in surface waters. *J Appl Microbiol* 2009;106:572-579.

contamination with these pathogens, which has in some cases been hampered by a lack of quantitative data. The data appear to indicate that the water quality of ponds in watersheds containing animal agriculture is improved by decreasing FIB, but not necessarily the pathogen loads. For example, pond inflow concentrations of *Salmonella* were generally but not always associated with high *E. coli* and fecal enterococci concentrations. The data collected by monitoring surface waters for fluxes of these contaminants could aid in the development of BMPs to reduce them.

Ponds can serve in wastewater treatment as a form of tertiary treatment or "polishing" of water leaving sewage treatment systems. Ponds have long retention times and serve to decrease biological oxygen demand (BOD) loads through long-term physical capture and biological degradation of contaminants.[193] They also have a tendency to improve ecological diversity by providing multiple and improved niches for wildlife. If managed properly and not overloaded with coliforms, they can markedly improve the quality of water that leaves the ponds and enters the watershed of interest. The downside to ponds is the potential for natural impact of coliforms from migratory birds. At this time, there is no means of separating whether the coliforms present in waterways, as tested by routine EPA methods, are coming from domestic or wildlife species. Thus, large flocks of migratory birds can add copious coliform counts to fields and associated waterways and could implicate farm animals as a major source of impaction, when in reality it may simply be a measure of a healthy ecosystem supporting a large number of visiting species.

Even a site-based intervention as simple as the avoidance of spreading manure on hydrologically-active areas (also known as hydrologically sensitive areas, HSAs), which are those areas deemed most likely to generate runoff, could have significant mitigative effects. HSAs can be easily delineated with hydrologic models, and real-time, forecast-based, web-based mapping showing areas where saturation (and thus runoff) are most likely to occur in the forecast period are under development.[194]

---

[193] BOD is an indirect measure of the amount of organic matter in a water body or pollutant that consumes oxygen as it is degraded by natural microorganisms. It is not directly related to pathogens, but BOD reduction may correlate to reduction in the amount of waste present.

[194] Cornell University Soil and Water Lab Web site. Hydrological sensitive areas – Salmon Creek. Available at: http://www.hsadss.bee.cornell.edu/Website/SalmonCreek/viewer.htm

6.7 Regulation

Federal regulations with regard to pathogens in water systems are intended to reduce potentially harmful pathogen inputs and to protect the public from exposure to such pathogens. In the former case, dischargers of waste products are required to obtain a permit under the National Pollutant Discharge Elimination System program, which specifies quantity and quality limitations on discharges. For animal operations, specific guidelines are available under the "CAFO" Rule, which was first published in 2003 and then revised in 2008.[195] Under the authority of the Clean Water Act (CWA), these rules establish permitting requirements and effluent limitation guidelines and standards for CAFOs to ensure proper manure management. The CWA is thus an environmental law intended to impact water quality by helping to set water quality standards. It also helps facilitate setting TMDLs; it is thus through this law that non-point sources can be impacted and coliform limits can be set.

With regard to public exposure to pathogens, regulations are in place for contact exposure such as through recreation and exposure via ingestion. Contact exposure is limited through the Beaches Environmental Assessment and Coastal Health Act of 2000,[196] which includes monitoring requirements and requirements for beach closings based on indicator organism concentrations. Drinking water exposure is regulated through a series of drinking water regulations that limit the concentrations of pollutants in drinking waters and require the use of certain treatment practices for certain water systems. Monitoring regulations are also in place to ensure safety of water. While a discussion of all water regulations is beyond the scope of this report, the following regulations are highlighted as they pertain to the control and mitigation of pathogens in water systems. All of the following were implemented under the authority of the Safe Drinking Water Act.

1. <u>Total Coliform Rule</u>
   The Total Coliform Rule, which applies to both surface and groundwater, sets health goals and legal limits for the presence of total coliforms in drinking

---

[195] 40 CFR Parts 9, 122, and 412, Revised National Pollutant Discharge Elimination System Permit Regulation and Effluent Limitations Guidelines for Concentrated Animal Feeding Operations in Response to the Waterkeeper Decision; Final Rule.

[196] United States Environmental Protection Agency Web site. Beaches Environmental Assessment and Coastal Health Act of 2000. Available at http://water.epa.gov/lawsregs/lawsguidance/beachrules/act.cfm. Accessed September 6, 2010.

water, and details the type and frequency of testing that water systems must undertake.[197] The rule, which became effective in 1989, is under revision.

2. Surface Water Treatment Rules
   The Surface Water Treatment Rules (SWTRs)[198], which are comprised of four individual rules, aim to improve control of microbial contaminants (particularly viruses, *Giardia*, and *Cryptosporidium*). The rules apply to all public water systems using surface water or ground water under the direct influence of surface water, and require such systems to filter and disinfect.

3. Filter Backwash Recycling Rule
   The Filter Backwash Recycling Rule, published in 2001, establishes regulatory requirements that govern the way that certain recycle streams are handled within conventional and direct filtration water treatment systems, to prevent issues that may arise by recycling streams with high concentrations of pathogens[199]. The Rule also establishes reporting and recordkeeping requirements for recycle practices to enable evaluation of the impact of recycle practices on overall treatment plant performance.

4. Groundwater Rule
   The more recent Groundwater Rule, published in 2006, provides for increased protection against microbial pathogens in public water systems sourced by ground water.[200] This rule arose out of concerns about the susceptibility of groundwater systems to fecal contamination.

While regulations under the Clean Water Act strive to control the discharge of pathogens into water systems, they cannot completely eliminate such sources. The drinking water rules provide redundancy under the “multiple barrier approach” so that public health is protected. The multiple barriers include source water protection, engineered treatment, and protection of

---

[197] United States Environmental Protection Agency Web site. Total Coliform Rule. Available at http://www.epa.gov/safewater/disinfection/tcr/index.html. Accessed July 25, 2010.

[198] These rules comprise four regulations dating from 1989: Surface Water Treatment Rule (SWTR); Interim Enhanced Surface Water Treatment Rule (IESWTR); Long Term 1 Enhanced Surface Water Treatment Rule (LT1ESWTR); and Long Term 2 Enhanced Surface Water Treatment Rule (LT1ESWTR).

[199] United States Environmental Protection Agency. *Filter Backwash Recycling Rule: technical guidance manual.* EPA 816-R-02-014. Office of Ground Water and Drinking Water, December 2002.

[200] United States Environmental Protection Agency Web site. Ground Water Rule. Available at http://www.epa.gov/safewater/disinfection/gwr/index.html. Accessed July 25, 2010.

treated water through the distribution system. All of these barriers work together to prevent transmission of pathogens to humans through water sources.

The Safe Drinking Water Act (SDWA) is significant because it gave EPA the authority to set regulations for drinking waters as discussed above. The SDWA also requires the EPA to publish a Contaminant Candidate List (CCL) every five years. This list represents contaminants not currently subject to any proposed or promulgated national primary drinking water regulations, that are known or anticipated to occur in public water systems, and which may require regulation under the SDWA. Thus, the list includes potential future threats for which more information is needed in order to make any regulatory decisions. Table 4 provides microbial contaminants on the CCL3, which was published in 2009 (the list also includes chemical contaminants, which are not provided here). Many of these agents on the CCL3 are the same pathogens that are included on our own "Pathogens of Potential Concern" list (Table 3). While practicality necessitates that stakeholders work to manage regulated microbes, our effort here has been forward-looking. We hope the ideas presented are sufficiently dynamic to encompass future threats such as these.

Table 4: Microbial contaminants on EPA's Contaminant Candidate List 3[201]

Microbe	Information	Typical Source	Other Sources, notes
Adenovirus	Virus most commonly causing respiratory illness, and occasionally gastrointestinal illness	Humans	Animal sources considered minimal
Caliciviruses	Virus causing mild self-limiting gastrointestinal illness; includes norovirus	Humans	Role of animal noroviruses under investigation
Campylobacter jejuni	Bacterium causing mild self-limiting gastrointestinal illness	Poultry feces	Most infections associated with incorrectly prepared meat

---

[201] United States Environmental Protection Agency Web site. Drinking water contaminant candidate list and regulatory determinations. Available at http://www.epa.gov/ogwdw000/ccl/ccl3.html. Accessed July 12, 2010.

Enterovirus	Group of viruses including polioviruses, coxsackieviruses and echoviruses that can cause mild respiratory illness	Humans	Animal sources considered minimal
Escherichia coli (O157)	Toxin-producing bacterium causing gastrointestinal illness and kidney failure	Cattle feces	Most infections from undercooked ground beef contaminated in processing.
Helicobacter pylori	Bacterium sometimes found in the environment capable of colonizing human gut that can cause ulcers and cancer	Humans	Pigs have their own species, *H. suis* and *H. heilmannii*
Hepatitis A virus	Virus that causes a liver disease and jaundice	Humans	
Legionella pneumophila	Bacterium found in the environment including hot water systems causing lung diseases when inhaled	Free-living facultatively pathogenic bacterium	
Mycobacterium avium	Bacterium causing lung infection in those with underlying lung disease, and disseminated infection in the severely immunocompromised	*M. avium* subspecies *hominis* causes most human cases	*M. avium* subspecies *avium* in birds, but also found in mammals, including deer
Naegleria fowleri	Protozoan parasite found in shallow, warm surface and ground water causing primary amebic meningoencephalitis	Free-living facultatively parasitic amoeba	Lethal to most vertebrate hosts
Salmonella enterica	Bacterium causing mild self-limiting gastrointestinal illness	Cattle and poultry feces	Most cases from food contaminated with feces in processing
Shigella sonnei	Bacterium causing mild self-limiting gastrointestinal illness and bloody diarrhea	Humans	

Concluding Remarks and Key Points

In this chapter, we returned to one of the key questions of the workshop – *what is the risk to water from agricultural sources?* Wild animals such as deer or large flocks of birds can add copious coliform counts to fields and associated waterways and will make the farm animals appear as a major source of impaction. And the picture, of course, can be more complicated than a single source. What is the potential for wild animals to become infected via contact with livestock manures, and transport pathogens to other livestock animals, waters, and humans? We are still unable to differentiate the source, and without that, cannot determine the proportional contribution of any one group. It is, therefore, a challenge to recommend mitigatory practices without a real understanding of underlying risk that would necessitate such practices.

Nevertheless, the use of multiple barriers is a necessary step to preventing transmission of pathogens off of farms. Much of the funded grant work supports this findings. Simple techniques like sunlight-mediated water treatment to reduce *E. coli*, lagoon treatment to enhance die-off of *Cryptosporidium*, and the relatively cost-effective implementation of grassland buffers may all have substantial impact.

To truly be of value, the efficacy of barriers must be assessed, such as via monitoring ammonia concentrations in lagoons. Bacteria, viruses, and protozoa themselves should also be tested for, although where that is financially not feasible, low-cost indicators like fecal coliforms could be used. Instating a voluntary tracking and response system for large producers could prove to be of considerable value. Emerging technologies, despite some limitations, appear to offer improved resolution and reduced analysis time for pathogen identification, and should be considered for further development.

Key recommendations from this chapter are:

1. The benefits/effectiveness of natural and manmade barriers and multi-barrier approaches requires further study;
2. Innovative technologies that can effectively reduce pathogenic contaminants from farms point and non-point sources should be developed.
3. The timing, duration, frequency, and location of grazing can be managed to minimize the risk of pathogen loading of surface water

sources by maintaining adequate vegetative cover and minimizing congregation of cattle in streams.

4. A risk-based approach for pathogen control should be adopted, through integration with water safety plans for specific watersheds, and depending on the uses of water in the watershed;
5. Different barriers that work for different stakeholders should be defined;
6. *Cryptosporidium* and noroviruses should be the next targets for serious study and determination of practices that minimize their loading into the watershed.

7

Chapter 7: The Path Forward

Humans have systematically shortened the spatial and temporal pathways that would naturally help in pathogen removal, thus allowing larger concentrations of viable pathogens to enter our streams, lakes, and drinking water systems. Filtration and disinfection naturally occurs as pathogens move across the landscape, being killed by UV light on the soil surface, adsorbing onto mineral particles and organic matter within the soil profile, or being consumed by microscopic predators in healthy soil food webs. All of this takes time and opportunities for removal increase the longer the time and the soil pathway. Humans have systematically truncated these pathways. Tile drains limit the functional soil profile to less than a few meters and macropores facilitate the transfer to the drains. Tile drains are designed to discharge into roadside ditches, which subsequently connect efficiently to streams. Thus agricultural fields may be located in the headwaters of a watershed, with seemingly miles of soil pathway for filtering processes to occur. In reality, however, pathogens spread on the soil surface may have less than a meter of soil with which to interact and, depending on precipitation timing, may literally end up in downstream drinking water supplies in only a few hours.

Despite this, we do know a lot about the pathogens that thrive in animal waste. We understand much about their biology, how they are transported, and how waste can be treated to remove them or at least to minimize their numbers. So while we present many knowledge gaps and recommendations for research in this report, it is with the intention that practical measures will still be taken based on the knowledge that we do have at the present time.

Considerations of where research should target its limited resources and in what ways producers could be optimally included in the process circled us back to the question we posed at the beginning: *What is the risk?*

7.1 Conclusions

We recognize that pathogens in the water supply cannot be studied in a vacuum. Any analysis must ultimately be performed in the context of other factors such as nutrient loading, hormones, and climate change. This synthesis represents one contribution to that endeavor.

Stewardship of the public health is fundamentally an interdisciplinary venture. It should come as no surprise, then, that this workshop was also an interdisciplinary venture. Microbiologists, farmers, environmental engineers, chemical engineers, veterinarians, physicians, lawyers, extension agents, policymakers, and many others came together to ask questions and find answers. In *A New Biology for the 21st Century*,[202] the National Academy of Sciences posited that the essence of the "New Biology" is integration: integration not just within the sciences, but among members of academia, government, and industry as well. How we embrace scientific information to address public and planetary health may be a defining element of our future.

We must also consider pathogen reservoirs and dynamics on a global scale. Waterborne pathogens are much more prevalent in the developing world than in the U.S. Global connectivity yields rapid dissemination of pathogens, making it important for the U.S. to contribute to controlling disease in the developing world.

It is this kind of integrated approach that we hope will help us chart the future of the watersheds program at NIFA.

7.2 Recommendations

What can we do, and what can we do better? What can we change, what should we change, and how can Agriculture be part of the solution? Addressing these questions led the participants to the following recommendations in several categories.

---

[202] National Academies of Science. *A New Biology for the 21st Century*. National Academies Press, 2009.

7.2.1 Resources for farmers

a. Best practices for manure management

Farmers and researchers alike still have many questions. Given its substantial volume and potential for impact, what should be done with manure? Should manure be treated as a waste product, or as a resource? It contains both pathogens and nutrients. How do we manage infectious organisms from farms? How do we apply BMPs to reduce pathogen loads? What management solution is best for minimizing pathogens? What are the options in the tool kit for managing manure?

What are effective ways of decreasing pathogens once they are in the soil? This could take the form of tilling, altering the soil pH, or adding predators or competitors. In France, some people are anecdotally using measures aimed at preventing soil compaction to promote populations of predatory microorganisms in the soil in order to decrease pathogen levels.

One approach would be to validate different BMPs under different management systems. Producers (who best understand their animals) could be permitted to determine what BMPs are appropriate for their system, and then receive credit for successful prevention or mitigation. Auditing would be employed to confirm that the BMPs used are, in fact, working.

Field BMPs (grassed waterways, buffers, wetlands) cannot be judged solely on performance with pathogens, as these were originally designed for sediment and nutrient manure. One effective BMP is manure storage, but building this storage capacity would be expensive. Incorporation of manure or manure injection is a valuable practice, as it reduces pathogen loss in runoff. Application of manure to frozen ground should be prohibited.

Most data indicate that composting effectively reduces pathogens, but this really reduces the nitrogen content and value as fertilizer, so there is a tradeoff. Nevertheless, pathogen management should always be considered when planning animal agricultural operations. Watershed management, land management, and complementary land uses could be effective at reducing pathogen export. Farm management practices and pathogen load reduction methods exist, but additional reduction methods are needed that are effective and affordable for farmers. Local, basic approaches generally are not enough to prevent pathogen problems at the watershed-scale.

In the Dou, Rankin, and Aceto work discussed earlier,[203] manure composting was found to be effective in deactivation of *Salmonella* Newport as well as *E. coli* O157:H7 killing them within a single day (data to be published). Manure treatment with pH below 4.0 also appeared effective. For a farm with animals infected with the multidrug-resistant pathogens, such manure management might be implemented prior to manure spreading.

Scientists, policymakers, and extension agents need more information. We have made the case in this document that we do not know exactly what pathogens we should be worried about, what pathways might result in harmful exposures, or exactly how to test for them. Once these questions are further answered, we would like to see the knowledge shared with farmers, who could make use of guidance to better assess overall pathogen loads, complementary land uses, appropriate density of different uses, etc. within watersheds. *To do this, the efficacy of current BMPs must be demonstrated.* The relative vulnerability of different locations for pathogen releases should be considered as a strategy for managing waterborne and food-borne disease transmission. These strategies may be politically difficult to implement, but may provide very cost-effective and efficient management options. University farms are well suited to lead the way in developing and demonstrating manure management BMPs, perhaps with a focus on controlling proximity to transport vehicles.

Perhaps BMPs should be thought of not as *best* management practices, since "best" will vary from farm to farm, but rather as *beneficial* management practices. Different systems produce different results and have different challenges. What works for one producer based on his location, type of operation, and financial considerations may differ from his neighbor; BMPs and regulations must allow flexible means for producer compliance.

b. Education

Many of the group's findings can be disseminated to producers through extension or other programs. The approach of a few grant recipients provides useful examples.

After studying the effects of grassland buffers on preventing pathogen transport off of cattle operations, Atwill, Tate, and Yates[204] conducted a number

---

[203] Dou Z, Rankin S, Aceto, H. Survival and transport of *E. coli* 0157:H7 and *Salmonella* Newport in manure and manured soils. Contract no.: 2007-35102-18243.

[204] Atwill ER, Tate KW, Yates MV. Efficacy of grassland buffers for reducing *Salmonella*, *Cryptosporidium parvum*, and rotavirus in rangeland runoff. Contract no.: 2006-35102-17193.

of workshops and seminars for land owners and the agricultural and regulatory communities regarding vegetated buffers and other management practices. Dr. Atwill spoke to the San Louis Obispo Cattleman's Association about waterborne pathogens and indicators in California waterways. Another talk was given at the Annual Beef Day and Trade Show at California State University on current issues in pathogens and rangeland water quality.

The work of Boehm[205] has been communicated with researchers at the Bodega Bay Marine Laboratory and the School of Veterinary Medicine at the University of California, Davis, and also on a local level with employees of Natural Resources Conservation Service in Half Moon Bay, CA and Santa Cruz, CA during a water quality management workshop. Dr. Boehm felt these discussions would be of value to these communities, as counties along the central California coast are increasingly concerned with nutrient and fecal pollution into their watersheds. Simply speaking with local communities encountered at the various research field sites is an informal but potentially effective way to relay valuable information.

Schneider, Walter, and Buckley[206] noted that efforts to change farm field tilling or other practices will require education, funding, and key stakeholder engagement – not just producers but county highway engineers, for example. The researchers have presented results to stakeholders at county highway staff conferences.

The Rogers and Shanks[207] work resulted in development of a new graduate-level course on livestock manure management at Clarkson University. Their goal was to translate information from the grant work and related projects to future agricultural and environmental engineers and scientists.

In this context of education, participants proposed a variety of topics for extension instruction:

---

[205] Boehm A. *Salmonella* and shiga-toxin encoding genes in coastal streams in Central California: relation to land use. Contract no.: 2007-35102-18139.

[206] Schneider RL, Walter MT, Buckley DH. Road ditch networks: rapid conduits for transporting pathogens and nutrients in agricultural runoff to drinking water supply systems. Contract no.: 2007-35102-18396.

[207] Rogers SW, Shanks O. Fate and transport of pathogenic microorganisms originating from livestock manures applied to agricultural lands. Contract no.: 2007-35102-18614.

Table 5: Extension course recommendations

- Conflicting BMPs
- Farm management for enteric diseases
- Hygiene and biosecurity in prevention of human infections
- Incentives for pollution prevention
- Management tools for small versus large farms
- Ongoing instruction for evolving disinfection practices
- Pathogens of importance
- Precautions to minimize run-off following manure application to land
- Proper disposal of waste from pre-weaned animals
- Rapid conduits (critical control points)
- Rare event preparedness
- Recruitment of stakeholders for demonstration projects of management or interventions strategies
- Recruitment of stakeholders for participation in data gathering
- Risks and benefits of application of raw manure to agricultural land
- Spreading to saturated or frozen ground may be problematic
- Water quality management
- Ways in which community stakeholders can get involved

c. *Economic incentivization and cost sharing*

Farmers should be provided with the tools they need – informational and otherwise – to conduct a responsible manure management system. The economic feasibility of this is very important. Incentives for pathogen control and waste management are likely to be an effective means of reducing pathogen loading in the environment.

Collins, Gillies, and Maille[208,209] examined ways to provide economic incentives to farmers to reduce agricultural pollution (in this case, nitrates) by way of a field experiment that tested an alternative to conventional government programs. The premise of the alternative was that farmers would be paid as a

---

[208] Collins AR, Gillies WN, Maille P. Farmers as producers of clean water: providing economic incentives for reducing agricultural non-point pollution. Contract no. 2006-35102-17261.

[209] Maille P, Collins A, Gillies N. Performance-based payments for water quality: experiences from a field experiment. *J Soil Water Conserv* 2009;64:85A-87A.

group based on the quantity and ambient quality of water flowing from their watershed. The farmers themselves decide what, if any, abatement action to take and how to allocate payments among themselves. Could a watershed group payment formula account for background levels of pollution? Could it elicit desired participation and abatement responses from farmers? Farmers were paid as a group based on the quantity and quality of water flowing from a single watershed in West Virginia. Nitrates and other gauges of pollution were used to measure water quality changes. At the outset, farmers were provided with technical assistance to develop a group strategy toward water quality improvements. This research was conducted under the premise that by paying farmers to produce clean water, water quality would be perceived as an opportunity, rather than a threat.

More than half of farmer households in the watershed participated during the first year, representing approximately 36% of the agricultural land in the watershed. Education level increased the likelihood of participation. Farmers who cultivated "prime" farmland participated at lower rates, a phenomenon attributed to farmer perceptions that their participation would introduce uncertainty into their farm income. Participating farmers showed a willingness and ability to work jointly to address nitrate runoff, and requested detailed water quality information to help them problem solve. Ultimately, participants agreed to provide over $12,000 in cost share funding for various mitigation practices, such as planting cover crops on corn fields constructing fencing barriers to exclude livestock from the stream, and installing a treatment wetland. Group-level actions provided evidence that farmers are willing and able to work jointly to address runoff: these farmers developed an allocation scheme to distribute payments among themselves, which included using 90% of each monthly payment to provide cost share support to farmers who wished to adopt runoff-reducing practices.

Can farmers make some money off of their resources, such as selling the energy produced by their digesters? Farmers can, of course, profit from selling electricity generated from manure digesters – that is already happening in California and elsewhere, especially in relation to large CAFOs. Specific economic incentives vary with ability to sell/net-meter electricity back to the grid and with tax credits and other incentives. Digestion also provides another benefit in that the residual product is less bulky, contains most of the original nutrients, and is either acceptably sterile or very easy to finish compost so that a pathogen-free product is produced. This product is then suitable for sale for a variety of purposes, including fertilizer. Some are also producing Class A biosolids with market value from their manures, using technologies targeted for pathogen reduction. But for the most part, the needed infrastructure (electrical or otherwise) is not yet in place to allow farmers to realize substantial profits from waste conversion.

While we support the development of economic incentives for farmers in principle, the implementation of management practices should obviously be based on a balance of multiple variables – effectiveness, environmental friendliness, cost, etc. Participatory models usually depend on either the presence of a regulatory "stick," or extensive monitoring to provide performance-based measures, or both, as addressed below.

d. Participatory models

What the Collins, Gillies, and Maille study highlights is that voluntary participation is indeed possible. The work of Dick, Moore, and Rajashekara[210] in the Sugar Creek Watershed of Ohio led not only to scientific findings, as summarized previously, but also to a participatory model of watershed management.[211] As an alternative to federal regulatory limits on pollution loading (or what the authors refer to as an "expert-driven" management model), the authors proposed an alternative approach that emphasizes a farmer-researcher partnership. Through case studies of collaborative efforts in the region, the authors found that the groups in the Sugar Creek have been successful because of the emphasis they place on the needs and values of local residents. They have addressed stream impairments and pollution remediation in a way such that both government agency goals *and* local community needs are met. Community groups participated at early planning stages to establish goals and timeframes. The authors state: "Today, most natural resource problems are social in origin resulting from social and economic structures of society, and consequently solutions for them require incorporating cultural and community-focused solutions that engage people."

Based on their analyses of these events, the authors devised an innovative model of non-point source pollution remediation that incorporated strategies to address farmer needs, while accounting for local and regional farm structures. The model has also been adapted for point source pollution remediation that involves nutrient trading. Additional research highlights land tenure as a variable that contributes to conservation program success,

---

[210] Dick WA, Moore RH, Rajashekara G. Upper Sugar Creek Watershed, Ohio: a model watershed for study of pathogen origin, fate and transport. Contract no.: 2007-35102-18217.

[211] Parker JS, Moore R, Weaver M. Developing participatory models of watershed management in the sugar creek watershed (Ohio, USA). *Water Altern* 2009;2:82-100.

emphasizing the need to incorporate farm-ownership attributes into collaborative planning schemes.[212]

7.2.2 Public education

Education materials that target grade levels K-12, undergraduate and graduate students, consumers, and agricultural entrepreneurs would provide a means for communicating best practices to all levels of stakeholders. These could take the form of teaching modules.

Table 6: Proposed teaching modules

- Agricultural hygiene practices
- Agriculture and water quality
- Case studies of watershed pathogens issues
- Coliphage and microbial source tracking
- Dose-response relationships
- Enteric virus isolation, characterization, and genomic analysis
- Explain loading, fate, and transport
- Exposure assessment, transmission
- The food chain from farm to plate: accounting for all stages of susceptibility to contamination
- Food safety
- Geographical information systems watershed spatial analysis
- Health outcomes: epidemiological investigations
- Importance of protozoan pathogens in humans and animals
- Major sources of protozoan pathogens
- Potential control measures for protozoan pathogens
- Principles of risk assessment
- Problem formulation
- Risk assessment – how is it done?
- Risk assessment and epidemiology of enteric viruses
- Risk characterization: integrating the pieces
- Risk communication
- Risk management
- Toolbox of best management practices
- Watershed protection

---

[212] Parker JS, Moore R, Weaver M. Land tenure as a variable in community based watershed projects: some lessons from the Sugar Creek Watershed, Wayne & Holmes County, Ohio. *Soc Nat Resour* 2009;9:815-833.

7.2.3 Databases

With the continued development of research, regulation, and best practices, it can be a challenge to keep track of what is needed, useful, and practical. The goal of this workshop and synthesis was to provide a forum to consolidate that information. But far more information has been generated than can be composed into a single document, and the information is also constantly growing and changing.

Participants therefore suggested that databases to collate information and provide an outlet for it would be of considerable value. Many databases already exist to monitor human disease outbreaks, and could be used as models. Driven by efforts to share, integrate, and synthesize available datasets, a number of possibilities were suggested:

1. A national pathogen occurrence/concentration database including information on manure, pathogen loads, surface water, ground water, soil, sediment, die-off, growth, and other rates.

2. A national sample database. Samples are collected for diverse purposes, and by many different agencies. Sample data could include data on water sources developed by water utilities and state agencies, as well as research data from government agencies, academia, and other research entities. Ideally this database would enable the tracing of outbreaks temporally and geographically, while also providing strain data. This would mitigate variation in state-to-state variation in coordination of epidemiologic studies in humans.

3. Collaboration to develop database on animal counts and land use activities to understand connection between land use, human health, and environmental contamination

4. A USDA-hosted website containing published literature on scientific methods of pathogen/watershed research.

A mechanism should also be established to ensure public access to existing databases obtained for diverse regulatory purposes. Current data collection efforts focus primarily on major waterways and drinking water sources.

7.2.4 Oversight, regulation, and guidance

There are many laws and regulations governing water and air quality, and farmers are often faced with the challenge of needing to adapt to myriad policies, some of which may conflict with one another. While it is beyond the scope of this report to assess the efficacy of these myriad policies, participants described what they viewed as a sensible, efficacious, integrated regulatory scheme.

Participants devised a regulatory scheme based upon the premise that decreasing pathogen load into water and foodstuffs in turn would decrease human disease. Regulators could target watersheds with known or high likelihood of contamination. Using a source reduction approach, a monitoring and regulatory system could be devised employing data collection (which would feed into future risk-based analyses), incentives as well as disincentives, and site- or region-specific implementation. Incentives for compliance could include cost-sharing or protection from lawsuits (the "blame" factor). Accountability is the goal. Credits for log reductions in pathogens could be offered, much the way the EPA offers log reduction credits for meeting treatment or other requirements. On-site practices and/or runoff would be monitored to assess the outcome of the interventions. So, there would be some monitoring at farm level and some at system level; some at source level and some at 'exposure site' level. Continued monitoring with a feed-back loop for refinement of the implemented measures is key, as should be state involvement, which will be the driver for successful implementation. A combination of good science and good regulation will be reflective of measured risk and common sense.

Risk analysis comprises risk assessment, risk management, and risk communication. Assessment informs degree of risk, shows where data gaps exist, and allows determination of the minimum levels of treatment (which are required to achieve the acceptable risk). The assessment then informs proposed risk management practices. Because our knowledge of pathogens and the risks they pose changes over time, this justifies the need for iterative, feedback loop-driven regulation. Once the risk has been assessed through scientific study, risk management practices (voluntary or regulated) can be undertaken. All of it must be communicated appropriately to stakeholders. An emphasis on the link between animal health/pathogen shedding and human/public health could help emphasize the need for on-farm pathogen reduction. But again, we must determine those pathogens about which we are concerned.

It was emphasized that top-down regulation is not the only framework (and may or may not be the best) for implementing compliance with best practices. Federal *guidance* alone can be very valuable. An example would be the water re-use “minimum requirement guidance,” which does not have regulatory status, but is nevertheless employed by states to drive healthy practices. This idea of the federal government serving as the promulgator of minimum requirements that can be employed and amended by individual states given their specific needs, local climate, etc. could be very appealing.

An interesting example is a program run by the Natural Resources Conservation Service (NRCS) Environmental Quality Incentives Program. This program provides cost-share for agricultural producers to plan and implement a large variety of structural and non-structural environmental practices. An expansion of this already successful program to recognize and emphasize practices with public health and environmental benefits could increase the adoption of multi-barrier approaches. Although not targeted to individual landowners, the Clean Water Act’s Section 319 Nonpoint Source Management program provides funding to states, territories, and tribes to mitigate nonpoint source pollution. More programs such as these could be targeted to areas of greatest risk. The United States Geological Survey runs the National Water Quality Assessment Program to look at water in concert with this, providing monitoring and accountability. A possible setup would be guidance and/or regulation promulgated at the state level, with cost sharing by the USDA.

The matter of cost sharing could have commanded an entire workshop and report in itself. There is a huge body of knowledge on the implementation of BMPs at the landowner and watershed level, and how cost-sharing plays a role. NRCS is the most common technical provider on BMP implementation and cost-sharing. This document is not the place to go into great depth on how to facilitate implementation of cost-sharing, but we recognize that this has been and can continue to be a valuable means to an end.

Participants furthermore proposed the development of a formal USDA evaluation of the risk to human health from manure pathogens. The outcome of this evaluation would result in greater definition of research gaps, recommendations for data collection, and a refined risk assessment.

All of the regulation in the world is meaningless without good science to describe the sources of pathogens. It is recommended that the EPA improve its *E. coli* testing in order to determine how much of *E. coli* impairment originates from specific sources. Doing so would probably mean that testing would have to move from fecal coliforms to something that would allow typing. We must move away from simple coliforms to a method that allows typing to determine if the contaminating fecal-derived bacteria are from human, agricultural, or wild-animal sources. The problem remains that in an agriculturally "impaired" watershed, we cannot be certain as to the sources of the coliforms present in these waters.

7.3 Research

Participants proposed a variety of research gaps that could be addressed by further study. Because this report has been prepared by request of the USDA, most of these research proposals address the types of problems in which the USDA would be particularly interested; some, such as in human epidemiology or basic microbiology, are more likely targets for funding by other entities. As listed, these titles are essentially broken down by the topics of this report. They are presented as potential titles for research studies. Many relate to the "Gaps" identified throughout the report, although not all gaps have been proposed as research studies; the most pressing or important were chosen. Some will require original research; others may be feasible through comprehensive literature reviews. Although some of the titles are general, all can be targeted to particular pathogens, animals, and scales. A multi-disciplinary approach to all of these research goals would, in our view, result in the most useful data and conclusions.

7.3.1 Source, survival, and reservoirs

- Zoonotic potential of enteric caliciviruses
- Zoonotic potential of rotaviruses
- The ecology and evolution/emergence of zoonotic enteric viruses: farm-level and watershed-level surveys
- Field-derived survival and inactivation data of selected pathogens of manure
- Livestock contribution of *Cryptosporidium* sp. to rural watersheds
- Transport potential of antibiotic resistant manure-origin bacteria at field and watershed scales

- Survival times and infectivity of super-surviving fractions
- Pathogen behavior in environmental reservoirs and effects on transmission
- Factors influencing retention, re-release, and motility of agricultural pathogens
- Interactions between pathogen survival and mobility leading to potential for long-term pathogen release or release of subpopulations with relatively high or low infectivity and virulence
- Interactions between pathogen survival and mobility leading to release of subpopulations with relatively high or low infectivity and virulence
- Association between genotypic and phenotypic traits of selected pathogens via development of a microarray

7.3.2 Transport and fate

- Monitoring of a small watershed: impacts on household water quality from on-site septic tank influent and effluent compared with AFO-generated waste
- Use of introduced mollusks as a sentinel organism in intake lines of a water treatment plant
- Review of the utility of qPCR as a tool for transport experiment analysis
- Comparison of pathogen loads on certified organic versus non-organic farms
- Review of pathogen behavioral data and transport processes in groundwater [or other medium]
- Watershed-scale assessment of pathogen sources, survival, transport, and transmission through multiple pathways (soils, groundwater, runoff, overland flow, in-stream transport, sediment dynamics)
- Watershed-scale assessment of disease transmission risks and pathways

- Cross-comparison of the transport behavior of multiple pathogens of concern

- Transport dynamics of antimicrobial-resistant plasmids: how farm management practices influence their spread

7.3.3 Sampling, isolation, and identification

- Development of pathogen detection methods based on pathogen-specific metabolites

- Recommendations for on-farm pathogen sampling: organisms, methods, and ramifications for farmers

- Development of validated detection methods for protozoa in soil and water

- A gene ecology perspective of pathogens: ability of genes to find new hosts via horizontal gene transfer

- Presence of antibiotics in the environment: influence on the maintenance and development of antibiotic resistance genes

- Methods of sampling to capture the spatial presence of pathogens over time

- Choosing the optimal sample size for viral analysis

- Use of coliphages as a research tool for identifying water contaminants

7.3.4 Risk assessment, modeling, and monitoring

- Recommendations for formalization of risk assessment procedures

- Development of multi-scale, predictive model frameworks for pathogen transmission [also for hydrology, water quality, etc.]

- Spatially distributed, time series, quantitative observations of pathogen distribution in environmental systems to support model development and testing

- Development of remote, real-time samplers for research purposes
- Analysis of field-scale measurements and interventions in the context of risk assessment and cost-benefit calculations

7.3.5 Remediation and mitigation

- Impacts of climate change on recommendations for manure management and treatment
- Waste processing techniques effective for viral inactivation
- pH and inactivation/disinfection characteristics of enteric viruses
- Community analysis of viruses in sewage and manure: informing risk assessments and establishing a means of source tracking

7.3.6 Medicine and epidemiology

- Potential for the creation and maintenance of hepatitis E virus-free swine herds in the United States, and the impact of such a venture
- Role of vaccination of pigs against porcine forms of hepatitis E virus in mitigating spread to people
- Development of advanced in vitro tools for assessment of zoonotic capabilities of animal pathogens
- Seasonal variation in pathogen shedding
- Harnessing the One Health initiative to develop a nexus for information sharing of infectious disease surveillance epidemiology for humans and animal diseases
- Use of metagenomic analysis to identify viruses in cattle, swine, and poultry manure
- Non-point source testing to assess runoff-associated risks to watersheds
- Air-facilitated dispersal of manure pathogens

- A study in viral evolution and emergence – recombination and reassortment capacities of zoonotic livestock pathogens

7.3.7 Management

- Best management practices for on-farm virus inactivation: an investigation of thermophilic processes, composting, ammonia, and high pH treatments

- Risk and cost-benefit analysis of common manure management processes

7.4 Climate change and other emerging threats

The system for controlling the better-known pathogens appears to work well. There is a need, however, for development of an effective and efficient response system to the new emerging pathogens and threats.

Ongoing identification of new viruses is needed. The development of methods that allow the relatively easy molecular identification of viral pathogens without the need to specifically isolate the viruses in animals or cell culture has allowed a dramatic increase in our ability to detect many types of viruses. This has also allowed much easier identification of their emergence and spread between hosts and within the environment. Although those viruses transmitted by insects may be dramatically impacted by any effects of global warming, it is much more difficult to predict what the effects would be on viruses transmitted via a fecal-oral route. However, new assays to distinguish infectious/non-infectious viruses are also needed to gauge the impact of disinfection/inactivation procedures.

Climate models predict greater frequency of extreme precipitation events and greater temperature fluctuations for large portions of the U.S. Extreme precipitation events are known to be directly related to pathogen outbreaks. The increased runoff from storm events enters drinking water systems, raising contaminant levels above thresholds for proper removal and disinfection. Large events can swamp both water and sewage treatment plants, cause treatment bypass at sewage treatment plants, overload manure holding lagoons, and cause high turbidity at drinking water plant intakes. In severe events, such as following hurricanes or similar heavy storms, it may be necessary to invoke extreme measures including large scale chlorination or boil water advisories.

Differences in temperature will shift pathogen sources, survival, and ecological dynamics. Additional emerging and re-emerging diseases should be expected. Shifting patterns of vectors and environmentally transmitted pathogens will likely be seen. Increased variability in precipitation should be expected. More extreme hydrologic events (floods and droughts) are likely to contribute additional pathogen transmission events and outbreaks.

The increased frequency of extreme events predicted from a changing climate will affect manure management practices, due to increased runoff, vectors, and pathogen emergence. Planning becomes exceedingly difficult in terms of being able to design for major weather-related impacts. On a positive note, the drive to reduce greenhouse gas emissions could drive increased manure recycling for clean energy.

Appendix A: Workshop participants

Participant	Affiliation	Expertise
Angenent, Lars	Cornell University	Pathogens in high-exposure human environments
Atwill, Edward R.	University of California, Davis	Efficacy of grassland buffers for reducing *Salmonella*, *Cryptosporidium parvum*, and rotavirus in rangeland runoff
Ballweber, Jeff	Pickering Inc.	Water regulatory issues
Bastian, Bob	U.S. Environmental Protection Agency	Water regulation
Boehm, Alexandria B.	Stanford University	*Salmonella* and Shiga-toxin encoding genes in coastal streams in central California: relation to land use
Bowman, Dwight D.	Cornell University	Survival kinetics of *Cryptosporidium* oocysts in swine facility wastes of the Southern Piedmont and Coastal Plain Watersheds
Bradford, Scot Alan	USDA Agricultural Research Service	A new paradigm for pathogen transport and deposition: the role of pore structure and colloid-colloid interactions
Burton, Alexandra	University of Georgia	Equine pathogens and molecular characterization
Carlin, Ellen	Carlin Consulting	Animal diseases and public health
Chen, Gang	Florida State University	*S. typhimurium* and *E. coli* O157:H7 transport modeling for agricultural practices
Coats, Eric	University of Idaho	Use of microarrays and qPCR/RT-qPCR for characterization of viral populations with water supplies affected by agricultural activities
Cornwell, Emily	Cornell University	Virology – fish, aquaculture, watersheds
Cummings, Kevin	Cornell University	Epidemiology and Disease Transmission from Cattle
Czymmeck, Carl	Cornell University (Pro-Dairy)	Manure management
Darnault, Christopher	University of Illinois at Chicago	Surfactant-facilitated transport of *Cryptosporidium parvum* in soil
Debbie, Dorothy	Cornell University	Bacteriology

Participant	**Affiliation**	**Expertise**
Dick, Warren A.	The Ohio State University	Upper Sugar Creek Watershed, Ohio: a model watershed for study of pathogen origin, fate, and transport
Dietert, Rod	Cornell University	Environmental Insult to the immune system
Dou, Zhengxia	University of Pennsylvania	Survival and transport of *E. coli* O157:H7 and *Salmonella* newport in manure and manured soils
Dubovi, Ed	New York State Diagnostic Laboratory	Virology
Earnest-Koons, Kathy	Cornell University	Pathology, swine disease
Eisenberg, Joseph	University of Michigan	Risk assessment and public health
Falbo, Kim	Cornell, DNR	Watershed management
Fayer, Ron	USDA Agricultural Research Service	Protozoan pathogens
Gerba, Charles	University of Arizona	Viruses in the environment
Ghiorse, William	Cornell University	Pathogen transport and interaction with soils
Gooch, Kurt	Cornell University (Pro-Dairy)	Dairy facility and waste management engineer
Gordon, Katrina	University of South Florida	Predicting the effects of agricultural practices on waterborne human pathogens, livestock helminths, and the health of rural water-bodies
Griffiths, Jeffrey K.	Tufts University	Infectious Diseases and their transmission as related to water, wastewater, and manure
Gouli, Vladimir	University of Vermont	*Escherichia coli* and *Cryptosporidium* occurrence, transport, fate and reduction from dairy farm point and non point pollution sources
Harter, Thomas	University of California, Davis	Human health risks from animal agriculture: comparative analysis of the transmission of multiple zoonotic pathogens in mixed-use agriculture

Participant	**Affiliation**	**Expertise**
Haznedaroglu, Berat	University of California, Riverside	Photochemical disinfection of pathogens: influence of extracellular polymeric substances on bactericidal capacity of reactive oxygen species
Hill, Jane	University of Vermont	Fate of motile bacterial pathogens in the subsurface: determining the extent and influence of motility and counter-current movement on cells
Houde, Alain	Agriculture & Agri-Food Canada	Biological Safety Officer
Jameson Jordan, Antonia	Cornell University	Veterinary Medicine
Jenkins, Michael B.	USDA Agricultural Research Service	Role of Ponds in reducing the threat of pathogen contamination form livestock in agricultural watershed
Jin, Yan	University of Delaware	Effect of land application of wastes on the fate and transport of pathogens in soil
Kravchenko, Sasha	Michigan State University	Transport and survival of Escherichia coli within soil aggregates
Kreher, Hal	Kreher's Eggs	Poultry management and manure composting
Lewis, Craig	University of Missouri	Beef cattle medicine
Lindsay, David S	Virginia Tech	Apicomplexan parasites in food and water
Liotta, Janice	Cornell University	Laboratory detection of pathogens
Lipp, Erin K.	University of Georgia	Watershed scale transport of *Salmonella*, *Campylobacter*, and indicator organisms in the Satilla River Basin
Lucio-Forster, Araceli	Cornell University	Pathogens in soil
Lucio-Martinez, Ben	Cornell University	Poultry management
Mark-Carew, Miguella	Cornell University	Pathogens/protozoa in manure
McEvoy, John	North Dakota State University	Source, fate, and transport of *Cryptosporidium* in a rural Midwestern watershed
McGovern, Cheryl	U.S. Environmental Protection Agency	Estuary protection

Participant	Affiliation	Expertise
Meals, Donald W.	Stone Environ Inc.	Assessment of dairy manure management practices to reduce pathogen runoff losses in agricultural watersheds
Meckes, Mark	U.S. Environmental Protection Agency	Bacterial detection and sampling
Mohammed, Hussni O.	Cornell University	Managing the risk of *Cryptosporidium* and *Salmonella* spp. in watersheds
Mohanram, Arvind	University of Hawaii	Influence of soluble organic matter on *Cryptosporidium parvum* oocyst motility in variable charge soils
Moore, Philip A.	USDA Agricultural Research Service	Quantification and control of bacterial pathogens in agricultural watersheds
Moorman, Tom	USDA Agricultural Research Service	Fate and significance of veterinary antibiotics in surface water
Morales, Veronica	Cornell University	Transport
Nguyen, Helen	University of Illinois	Investigating the spread of antimicrobial resistance near animal facilities: mechanisms of extracellular DNA transport and transfer
Nydam, Daryl	Cornell University	Dairy disease and management
Ospina, Paula	Cornell University	Dairy management
Packman, Aaron	Northwestern University	Environmental transport processes
Parker, John	Cornell University	Virology
Plummer, Jeanine D.	Worcester Polytechnic Institute	Water treatment and disease mitigation
Potter, Carolyn	Northeast Dairy Producers Association	Dairy management
Prodanovic, Masa	University of Texas at Austin	Quantifying the mechanisms of pathogen retention in unsaturated soils

Participant	**Affiliation**	**Expertise**
Rogers, Shane	Clarkson University	Fate and transport of pathogenic microorganisms originating from livestock manures applied to agricultural lands
Rozum, Mary Ann	USDA	Program leader, conservation & environment
Saif, L. J.	The Ohio State University	Noroviruses
Salvucci, Toni	Cornell University	Transport
Schneider, Rebecca	Cornell University	Road ditch networks: rapid conduits for transporting pathogens and nutrients in agricultural runoff to drinking water supply systems
Scidmore, Marci	Cornell University	Bacteriology
Seerley, Don	Pig producer	Swine manure management
Smith, James	U.S. Environmental Protection Agency (retired)	EPA regulations
Sobsey, Mark	University of North Carolina	Viruses and transmission
Steenhuis, Tammo	Cornell University	Assessing farm-source *E. coli* and *Salmonella* mobility in water
Szonyi, Barbara	Cornell University	Pathogens in the environment
Thurston, Jeanette	USDA	Conservation & environment
Timmes, Thomas	United States Military Academy	Transport and pathogen removal
Torres, Alfonso	Cornell University	Foreign animal diseases, biosecurity
Trotter, Karen	Cornell University	Bacteriology
Worobo, Randy	Cornell University	Chemical and genetic characterization of antimicrobial peptides (bacteriocins) produced by bacteria
Xiao, Lihua	Centers for Disease Control and Prevention	Molecular traceback of protozoal infections

Participant	**Affiliation**	**Expertise**
Yan, Tao	University of Hawaii	Survival of *Escherichia coli* on soil particle surface in Opaeula Watershed of Oahu, Hawaii
Zambriski, Jenny	Cornell University	Pathogens in manure
Zevi, Yuni	Cornell University	Transport of pathogens in soils
Zhang, Wu	Cornell University	Transport

Appendix B: NIFA-funded studies

USDA pathogen and water grants were funded beginning in 2005 through the National Water Program, a partnership between NIFA and land grant colleges and universities. The list below represents all such grant contracts represented in this paper. They were selected by accessing the program's Web site, http://www.usawaterquality.org/watershed/projects/default.html, and searching under "NIFA Agriculture and Food Research Initiative (Formerly NRI): Water and Watershed competitive grants." Projects from 2005 through 2009 (the last grant year prior to publication of this report) were scanned for those related to pathogens in the watershed. (One contract [Collins, Gillies, and Maille] was selected for its relevance to cost-sharing recommendations, even though the work related to nitrates and not pathogens.)

Atwill ER, Tate KW, Yates MV. Efficacy of grassland buffers for reducing *Salmonella*, *Cryptosporidium parvum*, and rotavirus in rangeland runoff. Contract no.: 2006-35102-17193.

Boehm, A. *Salmonella* and shiga-toxin encoding genes in coastal streams in Central California: relation to land use. Contract no.: 2007-35102-18139.

Bowman DD, Jenkins M, Sharpe R. Survival kinetics of *Cryptosporidium* oocysts in swine facility wastes of the Southern Piedmont and coastal plain watersheds. Contract no.: 2006-35102-17191.

Bowman DD. Current knowledge of sources, fate and transport of rural and agricultural watershed pathogens: a national synthesis workshop. Contract no.: 2009-65102-05842.

Bradford SA, Walker SL, Johnson B. A new paradigm for pathogen transport and deposition: the role of pore structure and colloid-colloid interactions. Contract no.: 2006-35102-17388.

Bryant SL. Quantifying the mechanisms of pathogen retention in unsaturated soils. Contract no.: 2007-35102-18162.

Chen G, Chan Hilton A. *S.* Typhimurium and *E. coli* O157:H7 transport modeling for agricultural practices. Contract no.: 2007-35102-18111.

Collins AR, Gillies WN, Maille P. Farmers as producers of clean water: providing economic incentives for reducing agricultural non-point pollution. Contract no. 2006-35102-17261.

Crawford RL. Use of microarrays and QPCR/RT-QPCR for characterization of viral populations within water supplies affected by agricultural activities. Contract no.: 2008-35102-04635.

Darnault C, Jacobson A. Surfactant-facilitated transport of *Cryptosporidium parvum* in soil. Contract no.: 2008-35102-19159.

Dick WA, Moore RH, Rajashekara G. Upper Sugar Creek Watershed, Ohio: a model watershed for study of pathogen origin, fate and transport. Contract no.: 2007-35102-18217.

Dou Z, Rankin S, Aceto, H. Survival and transport of *E. coli* O157:H7 and *Salmonella* Newport in manure and manured soils. Contract no.: 2007-35102-18243.

Drizo A, Gouli V. *Escherichia coli* and *Cryptosporidium* occurrence, transport, fate and reduction from Vermont dairy farm point and non-point pollution sources. Contract no.: 2008-35102-19222.

Fox G, Kanwar R, Malone R, et al. Role of directly connected macropores in pathogen transport to subsurface drainage. Contract no.: 2007-35102-18242.

Haggard BE. Fecal bacteria transport in Ozark streams. Contract no.: 2006-35102-17357.

Harter T. Human health risks from animal agriculture: comparative analysis of the transmission of multiple zoonotic pathogens in mixed-use agriculture. Contract no.: 2008-35102-18733.

Hill JE, Walker SL. Fate of motile bacterial pathogens in the subsurface: determining the extent and influence of motility and counter-current movement on cell. Contract no.: 2007-35102-18262.

Jenkins MB. The role of ponds in reducing the threat of pathogen contamination from livestock in agricultural watersheds. Contract no.: 2005-35102-16374.

Jin Y, Sims JT, Kniel K. Effect of land application of wastes on the fate and transport of pathogens in soil. Contract no.: 2006-35102-17405.

Jones A. Watershed scale assessment of a karst drainage basin using microbial, geospatial, and geochemical approaches. Contract no.: 2008-35102-19217.

Kravchenko A, Rose J, Smucker A. Transport and survival of *Escherichia coli* within soil aggregates. Contract no.: 2008-35102-04567.

Kuhlenschmidt M. Control of *Cryptosporidium* and rotavirus contamination in agricultural watersheds. Contract no.: 2006-35102-17344.

Lipp EK, Jenkins MB, Lowrance R, et al. Watershed scale transport of *Salmonella*, *Campylobacter*, and indicator organisms in the Satilla River Basin. Contract no.: 2006-35102-17328.

Mackie RI, Chee-Sanford J, Krapac, IG, et al. Antibiotic resistance genes and residues in water and soil in close proximity to swine production facilities. Contract no.: 2005-35102-16426.

McEvoy JM. Sources of *Cryptosporidium* in a rural North Dakotan river. Contract no.: 2006-35102-17237.

McEvoy JM, Clark ME, Khan E. Source, fate and transport of *Cryptosporidium* in a rural Midwestern watershed. Contract no.: 2008-35102-19260.

Meals DW, Braun D, Hanzas JP. Assessment of dairy manure management practices to reduce pathogen runoff losses in agricultural watersheds. Contract no.: 2006-35102-17197.

Mohammed HO, Chang YF, McDonough P, et al. Managing the risk of *Cryptosporidium* and *Salmonella* spp. in watersheds. Contract no.: 2006-35102-17356.

Nguyen TH, Zilles JL. Investigating the spread of antimicrobial resistance near animal facilities: mechanisms of extracellular DNA transport and transfer. Contract no.: 2008-35102-19143.

Noble RT. Understanding dynamics of microbial contaminant fate and transport in rural and agricultural lands. Contract no.: 2008-35102-04640.

Obropta CO, Miskewitz RJ, Phelps C. Investigation of the fate and transport of fecal contamination using microbial source tracking. Contract no.: 2007-35102-18145.

Ray C, Harvey RW, Chorover J. Influence of soluble organic matter on *Cryptosporidium parvum* oocyst mobility in variable charge soils. Contract no.: 2006-35102-17192.

Rogers SW, Shanks O. Fate and transport of pathogenic microorganisms originating from livestock manures applied to agricultural lands. Contract no.: 2007-35102-18614.

Russell JR, Ensley SM, Yoon KJ. Grazing management effects on pathogen loading of Midwestern pasture streams. Contract no.: 2007-35102-18115.

Schnabel WE, Barnes DL, Toniolo HA. Partitioning behavior of pathogen indicator microbes in springtime meltwater. Contract no.: 2008-35102-19215.

Schneider RL, Walter MT, Buckley DH. Road ditch networks: rapid conduits for transporting pathogens and nutrients in agricultural runoff to drinking water supply systems. Contract no.: 2007-35102-18396.

Steenhuis TS, Hay AG, Richards BK, et al. Visualization and quantification of bacterial transport in sand under steady and transient flow. Contract no.: 2005-35102-16316.

Steenhuis TS, Hay AG, Richards BK, et al. Assessing farm-source *E. coli* and *Salmonella* mobility to water. Contract no.: 2008-35102-04462.

Walker SL, Cwiertny DM. Photochemical disinfection of pathogens: influence of extracellular polymeric substances on bactericidal capacity of reactive oxygen species. Contract no: 2008-35102-04659.

Yan T. Survival of *Escherichia coli* on soil particle surface in Opaeula watershed of Oahu, Hawaii. Contract no.: 2009-35102-05212.

Appendix C: Publications resulting from NIFA-funded research

The research that led to the following publications was funded in full or in part by NIFA funds for the projects addressed in this report. Much of the grant-funded research is still in progress, so not all of the projects have yet resulted in publications.

Atwill ER, Tate KW, Yates MV. Efficacy of grassland buffers for reducing *Salmonella*, *Cryptosporidium parvum*, and rotavirus in rangeland runoff. Contract no.: 2006-35102-17193.

Tate KW, Atwill ER, Nader G, et al. Recent annual rangeland buffer and RDM study results. Proceedings for the Beef and Range Field Day: Sierra Foothill Research and Extension Center, University of California. April 19, 2007:21-24.

Tate KW, Atwill ER, Bedard-Haughn A, et al. Recent irrigated pasture water quality results. Proceedings for the Beef and Range Field Day: Sierra Foothill Research and Extension Center, University of California. April 19, 2007:25-30.

Bowman DD, Jenkins M, Sharpe R. Survival kinetics of Cryptosporidium oocysts in swine facility wastes of the Southern Piedmont and coastal plain watersheds. Contract no.: 2006-35102-17191.

Jenkins MB, Liotta JL, Lucio-Forster A, et al. Concentrations, viability, and distribution of *Cryptosporidium* genotypes in lagoons of swine facilities in Southern Piedmont and Coastal Plain watersheds of Georgia. *Appl Environ Microbiol* 2010;76:5757-5763.

Bradford SA, Walker SL, Johnson B. A new paradigm for pathogen transport and deposition: the role of pore structure and colloid-colloid interactions. Contract no.: 2006-35102-17388.

Bradford SA, Torkzaban S, Walker SL. Coupling of physical and chemical mechanisms of colloid straining in saturated porous media. *Water Res* 2007;41: 3012-3024.

Bradford SA, Toride N. A stochastic model for colloid transport and deposition. *J Environ Qual* 2007;36:1346-1356.

Bradford SA, Torkzaban S. Colloid transport and retention in unsaturated porous media: A review of interface, collector, and pore scale processes and models. *Vadose Zone J* 2008;7:667-681.

Bradford SA, Torkzaban S, Leij F, et al. Modeling the coupled effects of pore space geometry and velocity on colloid transport and retention. *Water Resour Res* 2009;45:W02414, doi:10.1029/2008WR007096.

Bradford SA, Kim HN, Haznedaroglu BZ, et al. Coupled factors influencing concentration dependent colloid transport and retention in saturated porous media. *Environ Sci Technol* 2009;43:6996-7002.

Johnson WP, Li X, Tong M, et al. Comment on Torkzaban et al (2008), Transport and fate of bacteria in porous media: coupled effects of chemical conditions and pore space geometry, *Wat Resour Res* 2009;44. W04403, doi:10.1029/2007WR006541.

Johnson WP, Pazmino E, Ma H. Direct observations of colloid retention in granular media in the presence of energy barriers, and implications for inferred mechanisms from indirect observations. *Water Res* 2010;44:1158-1169.

Kim HN, Walker SL. *Escherichia coli* transport in porous media: Influence of cell strain, solution chemistry, and temperature. *Colloids Surf B Biointerfaces* 2009;71:160-167.

Kim HN, Bradford SA, Walker SL. *Escherichia coli* O157:H7 transport in saturated porous media: Role of solution chemistry and surface macromolecules. *Environ Sci Technol* 2009;43:4340-4347

Kim HN, Hong Y, Lee I, et al. Surface characteristics and adhesion behavior of *Escherichia coli* O157:H7: Role of extracellular macromolecules. *Biomacromolecules* 2009;10:2556-2564.

Kim HN, Walker SL, Bradford SA. Macromolecule mediated transport and retention of *Escherichia coli* O157:H7 in saturated porous media. *Water Res* 2010;44:1082-1093.

Kim HN, Walker SL, Bradford SA. Coupled factors influencing the transport and retention of *Cryptosporidium parvum* oocysts in saturated porous media. *Water Res* 2010;44:1213-1223.

Ma H, Pedel J, Fife P, et al. Hemisphere-in-cell geometry to predict colloid deposition in porous media. *Environ Sci Technol* 2010;43:8573-8579.

Ma H, Johnson WP. Colloid retention in porous media of various porosities: Predictions by the hemispheres-in-cell model. *Langmuir* 2010;26:1680-1687.

Tazehkand SS, Torkzaban S, Bradford SA, et al. Cell preparation methods influence E. coli D21g surface chemistry and transport in saturated sand. *J Environ Qual* 2008;37: 2108-2115.

Tong M, Ma H, Johnson WP. Funneling of flow into grain-to-grain contacts drives colloid-colloid aggregation in the presence of an energy barrier. *Environ Sci Technol* 2008;42:2826-2832.

Torkzaban S, Bradford SA, Walker SL. Resolving the coupled effects of hydrodynamics and DLVO forces on colloid attachment to porous media. *Langmuir* 2007;23:9652-9660.

Torkzaban S, Bradford SA, van Genuchten MT, et al. Colloid transport in unsaturated porous media: The role of water content and ionic strength on particle straining. *J Contam Hydrol* 2008;96:113-127.

Torkzaban S, Tazehkand SS, Walker SL, et al. Transport and fate of bacteria in porous media: Coupled effects of chemical conditions and pore space geometry. *Water Resour Res*, 2008;44. W04403, doi:10.1029/2007WR006541.

Torkzaban S, Walker SL, Bradford SA. Reply to comment by William P. Johnson et al. on "Transport and fate of bacteria in porous media: Coupled effects of chemical conditions and pore space geometry", *Water Resour Res* 2009;45. W09604, doi:10.1029/2008WR007576.

Torkzaban S, Kim HN, Simunek J, et al. Hysteresis of colloid retention and release in saturated porous media during transients in solution chemistry. *Environ Sci Technol* 2010;44:1662-1669.

Chen G, Chan Hilton A. *S.* Typhimurium and *E. coli* O157:H7 transport modeling for agricultural practices. Contract no.: 2007-35102-18111.

Liu J, Subramaniam P, Martin L, et al. *E. coli* O157:H7 desorption by rhamnolipid biosurfactant in water-unsaturated porous media. *J Adhes Sci Technol* 2010, in press.

Chen G, Driks A, Tawfiq K, et al. *Bacillus anthracis* and *Bacillus subtilis* spore surface properties and transport. *Colloid Surface B* 2010;76:512-518.

Chen G, Subramanian P, Tawfiq K. Bacterial deposition in unsaturated porous media as related to surface properties. *Int J Environ Pollut* 2010;40:363-379.

Chen G. Bacterial interactions and transport in unsaturated porous media. *Colloid Surface B* 2008;67:265-271.

Penagonda Srinivasa Ranga V, Chan Hilton A, Chen G. Lipopolysaccharide extraction on bacterial adhesion and transport. *J Adhes Sci Technol* 2008;22:1073-1088.

Chen G, Penagonda Srinivasa Ranga V, Mao Y, et al. Impact of *lux* gene insertion on bacterial surface properties and transport. *Res Microbiol* 2008;159:145-151.

Chen G, Liu J, Tawfiq K, et al. Colloid retention in unsaturated porous media as impacted by colloid size. *Part Sci Technol* 2009;27:35-49.

Patil S, Zhang J, Tawfiq K, et al. Colloid interactions and transport in porous media as impacted by water chemistry. *J Adhes Sci Technol* 2009;23:1845-1859.

Collins AR, Gillies WN, Maille P. Farmers as producers of clean water: providing economic incentives for reducing agricultural non-point pollution. Contract no. 2006-35102-17261.

Maille P, Collins AR, Gillies N**.** Performance-based payments for water quality: experiences from a field experiment. *Water Net Resources.* Spring 2009:8-9. Available at: http://www.wvca.us/news/upload/wvwn_waternet/1649_2009%20Spring%20Water%20Net.pdf

Maille P, Collins A**.** Farmers as producers of clean water: a field experiment. In: Pascual U, Shah A, Bandyopadhyay J, eds. *Water, agriculture and sustainable well-being*. New Delhi: Oxford University Press, 2009:93-114.

Maille P, Collins A**.** *Performance-Based Payments for Conservation: Experience from a Water Quality Field Test with Farmers*. Saarbrucken, Germany: VDM Verlag Dr. Müller, 2009.

Maille P, Collins A, Gillies N**.** Performance-based payments for water quality: experiences from a field experiment. *J Soil Water Conserv* 2009;64:85A-87A.

Crawford RL. Use of microarrays and QPCR/RT-QPCR for characterization of viral populations within water supplies affected by agricultural activities. Contract no.: 2008-35102-04635.

Gregg M. Dairy waste stabilization and commodity production utilizing a two-stage anaerobic digestion process, in Civil Engineering. 2010, University of Idaho: Moscow. p. 68. Masters of Science thesis.

Dick WA, Moore RH, Rajashekara G. Upper Sugar Creek Watershed, Ohio: a model watershed for study of pathogen origin, fate and transport. Contract no.: 2007-35102-18217.

Merrick NN. Microbial source tracking: watershed scale study of pathogen origin, fate, and transport in the Upper Sugar Creek Watershed, Northeast Ohio. 2010, The Ohio State University: Columbus. PhD Dissertation

Moore R. Ecological integration of the social and natural sciences in the Sugar Creek method. In: Bohlen P. House G, eds. *Sustainable agroecosystem management*. Boca Raton: CRC Press, 2009;21-40.

Moore R. Linking the Social and Natural Sciences in Water Quality Trading in Ohio. *Tohoku J Agric Res* 2009;59:106-111.

Parker JS, Moore R, Weaver M. Developing participatory models of watershed management in the sugar creek watershed (Ohio, USA). *Water Altern* 2009;2:82-100.

Parker JS, Moore R, Weaver M. Land tenure as a variable in community based watershed projects: some lessons from the Sugar Creek Watershed, Wayne & Holmes County, Ohio. *Soc Nat Resour* 2009;9:815-833.

Ramirez NE, Wang P, Lejeune J, et al. Effect of tillage and rainfall on transport of manure-applied *Cryptosporidium parvum* oocysts through soil. *J Environ Qual* 2009;38:2394-2401.

Drizo A, Gouli V. *Escherichia coli* and *Cryptosporidium* occurrence, transport, fate and reduction from Vermont dairy farm point and non-point pollution sources. Contract no.: 2008-35102-19222.

Lee M, Drizo A, Rizzo D, et al. Evaluating the efficiency and temporal variation of pilot-scale hybrid and integrated constructed wetlands for treating high BOD and P concentrated dairy effluent. *Water Res* 2010;44:4077-4086.

Fox G, Kanwar R, Malone R, et al. Role of directly connected macropores in pathogen transport to subsurface drainage. Contract no.: 2007-35102-18242.

Fox GA, Kanwar R, Malone R. Earthworms and *E. coli*: a perilous combination for drain flow water quality. *Resource* 2008;15:22-24.

Garbrecht K., Fox GA, Guzman J, et al. *E coli*. transport through soil columns: implications for biorete ntion cell removal efficiency. *Trans ASABE* 2009;52:481-486.

Guzman J, Fox GA, Malone R, et al. *E. coli* transport from surface-applied manure to subsurface drains through artificial biopores. *J Environ Qual* 2009;38:2412-2421.

Haggard BE. Fecal bacteria transport in Ozark streams. Contract no.: 2006-35102-17357.

David MM, Haggard BE. Development of regression-based models to predict fecal bacteria numbers at select sites within the Illinois River Watershed, Arkansas and Oklahoma. *Water Air Soil Pollut* 2010, doi 10.1007/s11270-010-0497-7).

David MM. Development of regression-based models to predict fecal indicator bacteria at the Illinois River Basin, Arkansas and Oklahoma. 2008 University of Arkansas. Masters thesis.

Hill JE, Walker SL. Fate of motile bacterial pathogens in the subsurface: determining the extent and influence of motility and counter-current movement on cell. Contract no.: 2007-35102-18262.

Haznedaroglu BZ, Zorlu O, Hill JE, et al. Identifying the role of flagella in the transport of motile and nonmotile *Salmonella enterica* serovars. *Environ Sci Technol* 2010;44:4184-4190.

Jenkins MB. The role of ponds in reducing the threat of pathogen contamination from livestock in agricultural watersheds. Contract no.: 2005-35102-16374.

Jenkins MB, Endale DM, Fisher DS. Most probable number methodology for quantifying dilute concentrations and fluxes of *Salmonella* in surface waters. *J Appl Microbiol* 2008;104:1562-1568.

Jenkins MB, Endale DM, Fisher DS, et al. Most probable number methodology for quantifying dilute concentrations and fluxes of *Escherichia coli* O157:H7 in surface waters. *J Appl Microbiol* 2009;106:572-579.

Jin Y, Sims JT, Kniel K. Effect of land application of wastes on the fate and transport of pathogens in soil. Contract no.: 2006-35102-17405.

Attinti RJ, Wei K, Kniel JT, et al. Virus' (MS2, ΦX174 and Aichi) attachment on sand measured by atomic force microscopy and their transport through sand columns. *Environ Sci Technol* 2010, 44:2426-2432.

Wei J, Jin Y, Sims JT, et al. Survival of human Adenovirus 41 in land applied manure and biosolids. *Food Environ Virol* 2009; 1:148-154.

Wei J, Jin Y, Sims JT, et al. Attachment and internalization of murine norovirus-1 in manure and biosolids to Romaine lettuce. *Appl Environ Microbiol* 2010;76:578-583.

Wei J, Jin Y, Sims JT, et al. Survival of murine norovirus and Hepatitis A virus in different types of manure and biosolids. *Foodborne Pathog Dis* 2010; doi:10.1089=fpd.2009.0490. (In press)

Jones A. Watershed scale assessment of a karst drainage basin using microbial, geospatial, and geochemical approaches. Contract no.: 2008-35102-19217.

Albright M. Assessment of agricultural best management practices in the brushy creek watershed. 2007, Eastern Kentucky University: Richmond. Masters of Science thesis.

Albright M. Assessment of agricultural best management practices in the brushy creek watershed. Eastern *Kentucky Environmental Research Institute Report Summary Series*, 2007; RS07.005.

Kravchenko A, Rose J, Smucker A. Transport and survival of *Escherichia coli* within soil aggregates. Contract no.: 2008-35102-04567.

Kravchenko AN, Martin MA, Smucker, A, et al. Theoretical and practical limitations in the determination of multifractal spectra from pore/solid soil aggregate images. *Vadose Zone J.* 2009;8:220-226.

Kuhlenschmidt M. Control of *Cryptosporidium* and rotavirus contamination in agricultural watersheds. Contract no.: 2006-35102-17344.

Janjaroen D, Liu Y, Kuhlenschmidt, MS, et al. Role of divalent cations on deposition of *Cyrptosporidum parvum* oocysts on natural organic matter surfaces. *Environ Sci Technol* 2010. (In press)

Janjaroen D, Liu Y, Kuhlenschmidt, MS, et al. Characterization of *Cryptosporidium parvum* oocyst wall macromolecules and adhesion kinetics of oocysts on quartz surface. *Biomacromolecules* 2010. (In press)

Liu Y, Janjaroen D, Kuhlenschmidt MS, et al. Deposition of *Cryptosporidium parvum* oocysts on natural organic matter surfaces: microscopic evidence for secondary minimum deposition in a radial stagnation point flow cell. *Langmuir* 2009;25:1594-605.

Pineda MF, Chan LL, Kuhlenschmidt TB, et al. Rapid label-free selective detection of porcine rotavirus using photonic crystal biosensors for groundwater monitoring. *IEEE Sensors J* 2008;9:470-477.

Schmidt J, Kuhlenschmidt MS. Microbial adhesion of *Cryptosporidium parvum*: identification of a colostrum-derived inhibitory lipid. *Mol Biochem Parasit* 2008;162:32-9.

Mackie RI, Chee-Sanford J, Krapac, IG, et al. Antibiotic resistance genes and residues in water and soil in close proximity to swine production facilities. Contract no.: 2005-35102-16426.

Chee-Sanford JC, Mackie RI, Koike S, et al. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *J Environ Qual* 2009;38:1086-1088.

Koike S, Aminov RI, Yannarell AC, Gans, et al. Molecular ecology of Macrolide-Lincosamide-Streptogramin B Methylases in waste lagoons and subsurface waters associated with swine production. *Microb Ecol* 2009;59:487-498.

Aminov RI, Mackie RI. Evolution and ecology of antibiotic resistance genes. *FEMS Microbiol Lett* 2007;271:147-161.

Koike S, Krapac IG, Oliver HD, et al. Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period. *Appl Environ Microbiol* 2007;73:4813-4823.

Jindal A, Kocherginskaya S, Mehboob A, et al. Antimicrobial use and resistance in swine waste treatment systems. *Appl Environ Microbiol* 2006;72:7813-7820.

Mackie RI, Koike S, Krapac I, et al. Tetracycline residues and tetracycline resistance genes in groundwater impacted by swine production facilities. *Anim Biotechnol* 2006;17:157-176.

McEvoy JM. Sources of *Cryptosporidium* in a rural North Dakotan river. Contract no.: 2006-35102-17237.

Feltus DC, Giddings CW, Khaitsa ML, et al. High prevalence of *Cryptosporidium bovis* and the deer-like genotype in calves compared to mature cows in beef cow-calf operations. *Vet Parasitol* 2008;151:191-195

Nguyen TH, Zilles JL. Investigating the spread of antimicrobial resistance near animal facilities: mechanisms of extracellular DNA transport and transfer. Contract no.: 2008-35102-19143.

Lu N, Zilles JL, Nguyen TH. Adsorption of extracellular chromosomal DNA and its effects on natural transformation of *Azotobacter vinelandii*. *Appl Environ Microbiol* 2010;76:4179-84.

Gutierrez L, Li X, Wang JW, et al. Adsorption of rotavirus and bacteriophage MS2 using glass fiber coated with hematite nanoparticles. *Water Res* 2009;43:5198-5208.

Gutierrez L, Mylon SE, Nash B, et al. Deposition and aggregation kinetics of rotavirus in divalent cation solutions. *Environ Sci Technol* 2010;44:4552-4557.

Janjaroen D, Liu YY, Kuhlenschmidt MS, et al. Role of divalent cations on deposition of cryptosporidium parvum oocysts on natural organic matter surfaces. *Environ Sci Technol* 2010;44:4519-4524.

Liu YY, Janjaroen D, Kuhlenschmidt MS, et al. Deposition of *Cryptosporidium parvum* oocysts on natural organic matter surfaces: microscopic evidence for secondary minimum deposition in a radial stagnation point flow cell. *Langmuir* 2009;25:1594-1605.

Mylon SE, Rinciog CI, Schmidt N, et al. Influence of salts and natural organic matter on the stability of bacteriophage MS2. *Langmuir* 2010;26:1035-1042.

Nguyen TH, Chen KL, M. Elimelech. Adsorption kinetics and reversibility of linear plasmid DNA on silica surfaces: influence of alkaline earth and transition metal ions. *Biomacromolecules* 2010;11:1225-1230.

Pham M, Mintz EA, Nguyen TH. Deposition kinetics of bacteriophage MS2 to natural organic matter: role of divalent cations. *J Colloid Interface Sci* 2009;338:1-9.

Ray C, Harvey RW, Chorover J. Influence of soluble organic matter on *Cryptosporidium parvum* oocyst mobility in variable charge soils. Contract no.: 2006-35102-17192.

Gao X, Chorover J. Adsorption of sodium dodecyl sulfate (SDS) at ZnSe and a-Fe2O3 surfaces: combining infrared spectroscopy and batch uptake studies. *J Colloid Interface Sci* 2010;348:167–176.

Gao X, Chorover J. In-situ monitoring of *Cryptosporidium parvum* oocyst surface adhesion using ATR-FTIR spectroscopy. *Colloids Surf B Biointerfaces* 2009;71:169-176.

Gao X, Metge DW, Ray C, et al. Surface complexation of carboxylate adheres *Cryptosporidium parvum* oocysts to the hematite/water interface. *Environ Sci Technol* 2009;43:7423-7429.

Mohanram A, Ray C, Harvey RW, et al. Comparison of transport and attachment behaviors of *Cryptosporidium parvum* oocysts and oocysts-sized microspheres being advected through three granular porous media. *Water Res* 2010. doi:10.1016/j.watres.2010.06.015.

Rogers SW, Shanks O. Fate and transport of pathogenic microorganisms originating from livestock manures applied to agricultural lands. Contract no.: 2007-35102-18614.

Donnelly M. 2009. Persistence of bacterial pathogens, fecal pollution indicators, and microbial source tracking markers in manure amended soils. 2009, Clarkson University Center for the Environment: Potsdam. Masters of Science thesis.

Liu D. Evaluation of Waterborne Pathogens Associated with a Concentrated Swine Feeding Operation in North Carolina. 2010, Clarkson University Center for the Environment: Potsdam. Masters of Science thesis.

Narula R, Grimberg S, Rogers S, et al. Pathogen reduction and factors responsible for pathogen reduction in dairy farm operations treating agricultural waste (in review).

Owens LB, Bonta JV, Shipitalo MJ, et al. Effects of Winter Manure Application in Ohio on the Quality of Surface Runoff. *J Environ Qual* (accepted).

Rogers SW, Donnelly M, Peed L, et al. Decay of bacterial pathogens, fecal indicators, and real-time quantitative PCR genetic markers in manure amended soils (in review).

Russell JR, Ensley SM, Yoon KJ. Grazing management effects on pathogen loading of Midwestern pasture streams. Contract no.: 2007-35102-18115.

Bear DA, Cho Y-I, Russell JR, et al. Incidence of bovine enterovirus, coronavirus, and group a rotavirus, and concentration of total coliforms in Midwestern pasture streams. Iowa State University 2010 Animal Industry Report. R2532.

Haan MM, Russell JR, Davis JD, et al. Grazing management and microclimate effects on cattle distribution relative to a cool season pasture stream. *Range Ecol and Man* 2010. (In press.)

Schneider RL, Walter MT, Buckley DH. Road ditch networks: rapid conduits for transporting pathogens and nutrients in agricultural runoff to drinking water supply systems. Contract no.: 2007-35102-18396.

Falbo K, Schneider RL, Buckley D, et al. Submitted to *J Environ Qual* August 2010.

Steenhuis TS, Hay AG, Richards BK, et al. Assessing farm-source *E. coli* and *Salmonella* mobility to water. Contract no.: 2008-35102-04462;

Steenhuis TS, Hay AG, Richards BK, et al. Visualization and quantification of bacterial transport in sand under steady and transient flow. Contract no.: 2005-35102-16316.

Gao B, Steenhuis TS, Zevi Y, et al. Capillary retention of colloids in unsaturated porous media, *Water Resour Res* 2008;44:W04504, doi:10.1029/2006WR005332.

Morales VL, Zhang W, Gao B, et al. Impact of dissolved organic matter on colloid transport in the vadose zone: deterministic approximation of transport deposition coefficients from polymer coating characteristics. Submitted to *Water Res*.

Salvucci AE, Zhang W, Morales VL, et al. The impact of biofilm-forming potential and tafi production on transport of environmental *Salmonella* through unsaturated porous media. *Biologia* 2009;3:460-464. doi:10.2478/s11756-009-0102-y.

Zevi Y, Dathe A, Gao B, et al. Transport and retention of colloidal particles in partially saturated porous media: Effect of ionic strength, *Water Resour Res* 2009;45:W12403. doi:10.1029/2008WR007322

Appendix D: Gaps identified

1. We lack detailed knowledge of the zoonotic capabilities of noroviruses. [This gap was already identified and is currently a focus of the USDA's ongoing research effort. Under the NIFA AFRI initiative in Food Safety, grants are being provided for *Prevention, Detection, and Control of Food-borne Viruses in Food: A Focus on Noroviruses*. Proposals are being sought to develop studies to evaluate the occurrence and fate of noroviruses and other food-borne viruses in food to assess their potential risk to food safety and human health. We encourage studies funded under these grants to address the zoonotic potential of noroviruses as related to livestock and wildlife.]

2. Despite significant investment by human health agencies, we lack broad knowledge of the biology, epidemiology, and serology of HEV, in animals other than swine.

3. Scientists interested in performing monitoring for research frequently lack the budget for state-of-the-art equipment or for manpower for regular sampling. It would be helpful for USDA grants to allow sufficient funding for such purposes.

4. More research is needed on wildlife-transmitted pathogens and the proportion of waterborne pathogens attributable to wildlife.

5. There is insufficient epidemiological data on the importance of waterborne transmission of *T. gondii, B. coli, Blastocystis* sp., and microsporidia*,* and the sources of the latter three pathogens are not well understood.

6. The antigenic and genetic relatedness between human and animal enteric viruses needs to be assessed, with special attention to noroviruses and rotaviruses.

7. Field-based, or highly realistic laboratory-based, studies of pathogen survival would add value to the understanding of survival and formation of nidi of infection within different environmental conditions. ("Nidi" (plural of nidus) are here defined as those places where a pathogen originates, is fostered, or develops; specifically, the points of origin or focus of an infection or disease process. These environmental nidi are distinct from "animal reservoirs," an animal host or passive carrier of a pathogen.)

8. Comprehensive assessments and comparisons of survival in diverse media (e.g., soil, water, manure) are needed for understudied pathogens, including *Blastocystis* sp. and microsporidia, about which little is known. The survival dynamics of even those pathogens that have been extensively studied, such as *E. coli* O157: H7 and rotavirus, are still not entirely elucidated.

9. The viability of enteric viruses when sequestered in manure, soil, or organic materials has not been well studied.

10. The biological mechanisms allowing bacteria to enter a persistent state are not well understood. Furthermore, how bacteria in persistent states (e.g., VBNC) regain activity is not understood.

11. The scientific community lacks surrogates that would allow field testing of survival models, such as the use of bacteriophages for enteric viruses. The surrogates would first have to be benchmarked against the pathogens of interest prior to development.

12. The scientific community should develop models of pathogen survival that incorporate factors contributing to the long-term fate of super-surviving subpopulations, and the effects of climate change.

13. Interactions between pathogens, soils, sediments, fecal matter, and organic matter are not well understood.

14. We do not understand pathogen behavior well enough in a comprehensive fashion to thoroughly evaluate risks.

15. The coupling between flow and sediment is not well understood. The relative role of direct transmission of pathogens in water vs. release and transport of pathogens associated with soils and sediments is not well understood.

16. Data are needed on transport of pathogens in man-made irrigation systems.

17. The risk posed by resistant pathogens is not entirely understood. More information is needed to quantify not only the development of antibiotic resistance on farms, but also the amount environmental transmission of resistant bacteria from production facilities and pastures, and the extent to which different farm management practices mitigate this transport.

18. There is no reliable source tracking technique for any pathogen.

19. Alternative quantitative methods to fecal coliforms as indicator organisms should be developed.

20. Sampling method limitations are an issue in terms of sensitivity, specificity, extraction efficiency, cost, limits of detection, and time for identification.

21. Sampling methods are cumbersome and time consuming. Improved technology for real-time in situ detection of indicators and/or specific pathogens would greatly improve our ability to understand linkages between pathogen sources, environmental reservoirs, and human infection.

22. Sampling methods are only standardized for *Salmonella*.

23. On-farm sampling methods are not overseen, and farmers are not necessarily provided with guidance on what method to use or how to apply it. The development of simple, farm-side tests may be of value both practically to the farmer and for epidemiological/research studies.

24. Improved hollow fiber ultrafilters are needed for enhanced virus recovery. In addition, virus recovery efficiencies should be established for these filters.

25. The extraction step prior to molecular methods needs to be optimized.

26. Employment of different methods can confer huge variability, making comparisons between datasets difficult.

27. Current methods may miss pathogens that are present but below the detection limit of the method.

28. Inexpensive and rapid assays to confirm virus infectivity are needed.

29. There are no standardized methods for purification and identification of many protozoa from a variety of different media.

30. PCR inhibitors are a ubiquitous problem and can bias extraction/detection procedures, especially if appropriate and validated internal controls are not used. What the inhibitors are, and what effects they have on PCR, are incompletely known.

31. Standardized methods for molecular-based approaches to pathogen identification are lacking.

32. Conceptual models to link pathogen source, genotype, transmission dynamics, and microbial ecology are lacking.

33. Soil extraction methods should be optimized such that qPCR is a feasible means of study for transport experiments.

34. Somatic coliphages as a tool for microbial source tracking should be investigated further.

35. Improved predictive models are needed to identify vulnerabilities arising from rare events, non-obvious interactions, multi-scale processes, and multiple barrier failures.

36. Different pathways require different models, with many challenges occurring at the interfaces of pathways. The need for integration of chemistry, biology, physics, and hydrology is an added challenge.

37. Producers lack practices for pathogen reduction from AFO point and non-point sources. A lack of understanding of basic biology and transport (as described earlier), and field testing and performance results, hampers development and refinement of practices.

38. There is a lack of understanding of pathogen retention mechanisms as they relate to BMPs.

39. Additional research is needed on the effects that different management practices have on pathogen transport. The extremes are better elucidated than the intermediate practices and their resulting effects on pathogen transport.

Appendix E: Microbiological methods

Select EPA methods for the testing and monitoring of drinking water

Adapted from http://www.epa.gov/nerlcwww/online.html

VIRUSES AND COLIPHAGE

USEPA Manual of Methods for Virology — Twelve chapters. The individual chapters were prepared as ASCII text files to be viewed/printed with a monospaced font. It is planned to make them also available in PDF format in the future. The manual (EPA 600-4-84-013) describes in detail procedures for detecting viruses in environmental samples.

Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure (April 2001) (PDF) (40 pp, 463K) — This document (EPA 821-R-01-030) describes an enrichment procedure for detecting male-specific and somatic coliphage in groundwater and other waters.

Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure (April 2001) (PDF) (38 pp, 376K)- This document (EPA 821-R-01-029) describes an single agar layer procedure for detecting and quantitating male-specific and somatic coliphage in groundwater and other waters.

BACTERIA

Method 1605: *Aeromonas* in Finished Water by Membrane Filtration Using Ampicillin-Dextrin Agar with Vancomycin (ADA-V) (October 2001) (PDF) (26 pp, 443K) — This method (EPA 821-R-01-034) describes a performance-based membrane filtration technique for the detection and enumeration of *Aeromonas* species. This method uses a selective medium that partially inhibits the growth of non-target bacterial species while allowing *Aeromonas* to grow and be detected by the production of acid from dextrin fermentation (causing a pH change producing yellow colonies). The method will be proposed for use in EPA's data gathering and monitoring programs under the Unregulated Contaminant Monitoring Rule.

Method 1604: Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium) (September 2002) (PDF) (18 pp, 486K) — This document (EPA 821-R-02-024) is identical to the February 2000 version of the MI Agar Method (Membrane Filter Method for the Simultaneous Detection of Total Coliforms and *Escherichia coli* in Drinking Water, EPA-600-R-00-13), with one exception, the addition of MI Broth. The MI Agar Method was approved for use in compliance monitoring of drinking water and source water in support of the National Primary and Secondary Drinking Water Regulations (Federal Register, Vol. 64, No. 230, 1 December 1999, p.67450-67467) becoming effective 3 January 2000. On 6 November 2001, MI Broth was approved as a minor modification. This method, now designated as Method 1604, has been approved for use in monitoring ambient water (Federal Register, Vol. 68, No. 139, 21 July 2003, p.43272-43283) becoming effective 4 August 2003. Four color photos of coliform and *E. coli* colonies on MI agar are also included in Method 1604. The procedure is in the format of the Environmental Monitoring Management Council (EMMC). The method may also be viewed as it appeared upon 1993 publication in the journal Applied and Environmental Microbiology (Vol 59, p.3534-3544) PDF (11 pp, 2.2MB), (EPA 600-J-99-225) but it does not include photos.

Method 1603: *Escherichia coli (E.coli)* in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC) (September 2002) (PDF) (13 pp, 129K) — This method (EPA 821-R-02-023) combines information from a 1985 publication (Test methods for *Escherichia coli* and Enterococci in Water by the Membrane Filter Procedure, EPA 600-4-85-076) and a subsequent March 2000 manual (Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and *Escherichia coli*, EPA/821/R-97/004). Method 1603 is a revised membrane filter (MF) procedure, a single-step method that uses one medium, modified mTEC Agar, and does not require the transfer of the membrane filter to another medium or other substrate.

Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-ß-D-Glucoside Agar (mEI) (September 2002) (PDF) (14 pp, 125K) — This method (EPA 821-R-02-022) combines information previously published in the May 1997 version of Method 1600 (Membrane Filter Test Method for Enterococci in Water, EPA-821-R-97-004a) and a subsequent March 2000 manual (Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and *Escherichia coli*, EPA/821/R-97/004). Method 1600 is a revision of EPA's previous enterococci method, used since 1985 in ambient water quality monitoring. It reduces analysis time to 24 hours and improves analytical quality. The method has been validated in single- and multi-laboratory studies and has undergone peer review.

Method 1106.1: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus-Esculin Iron Agar (mE-EIA) (September 2002) (PDF) (16 pp, 806K) — This method (EPA 821-R-02-021) combines information from a 1985 publication (Test methods for *Escherichia coli* and Enterococci in Water by the Membrane Filter Procedure, EPA 600-4-85-076) and a subsequent March 2000 manual (Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and *Escherichia coli*, EPA/821/R-97/004). Method 1106.1 is a membrane filter (MF) procedure for the detection and enumeration of enterococci in water. The method incorporates the sequential use of a selective medium, mE agar, and a differential medium, EIA agar.

Method 1103.1: *Escherichia coli (E. coli)* in Water by Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC) (September 2002) (PDF) (17 pp, 471K) — This method (EPA 821-R-02-020) combines information from a 1985 publication (Test methods for *Escherichia coli* and Enterococci in Water by the Membrane Filter Procedure, EPA 600-4-85-076) and a subsequent March 2000 manual (Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and *Escherichia coli*, EPA/821/R-97/004). Method 1106.1 is a membrane filter (MF) procedure for the detection and enumeration of *E. coli* in water. The method incorporates the use of a selective and differential medium, mTEC agar followed by incubation with urea substrate medium.

Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and *Escherichia coli* (March 2000) (PDF) (49 pp, 350K) — This manual (EPA 821-R-97-004) describes four test methods for measuring bacteriological densities in ambient waters: the original and a revised method for detecting enterococci, and the original and a revised method for detecting *E. coli*. All four methods use a membrane filter procedure. The manual complements a training video on this topic but may be used independently. *(To obtain the video, see pg 49 of the manual.)*

PROTOZOANS

Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA (PDF) (76 pp, 1.2MB) — December 2005 Update — This method (EPA 815-R-05-002) is a performance-based method applicable to the determination of *Cryptosporidium* and *Giardia* in aqueous matrices. Method 1623 requires filtration, immunomagnetic separation (IMS) of the oocysts and cysts from the material captured, and immunofluorescent assay (FA) for determination of the oocyst and cyst concentrations. A Training module is also available.

Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA (PDF) (75 pp, 1.3MB) — December 2005 Update — This method (EPA 815-R-05-001) is a performance-based method applicable to the determination of *Cryptosporidium* in aqueous matrices. Method 1622 requires filtration, immunomagnetic separation (IMS) of the oocysts from the material captured, and immunofluorescent assay (FA) for determination of the oocyst concentration. There are also forms associated with Method 1622. A training module is also available.

Appendix F: Additional suggested resources

General resources

Rosen BH. *Waterborne Pathogens in Agricultural Watersheds*. United States Department of Agriculture, Natural Resources Conservation Service. 2000. [Note: An updated edition of this report, authored by Edward R. Atwill, is undergoing review.]

Rogers S, Haines J. *Detecting and mitigating the environmental impact of fecal pathogens originating from confined animal feeding operations: review.* United States Environmental Protection Agency. 2005.

Smith Jr. JE, Millner PD, Jakubowski W, et al., eds. *Contemporary perspectives on infectious disease agents in sewage sludge and manure*. The JG Press, Inc., 2005.

United States Environmental Protection Agency, 2004. Risk Assessment Evaluation for Concentrated Animal Feeding Operations.

Waterborne Pathogens Web site. Available at: http://waterbornepathogens.org/

Liew D, ed. *Molecular detection of human viral pathogens.* Boca Raton: CRC Press, 2010. [The first in a series of four books to be published on viral, bacterial, fungal, and parasitic pathogens.]

Liu D, ed. *Molecular detection of foodborne pathogens*. Boca Raton: CRC Press, 2010.

Resources on bovine somatotropin

Capper JL, Castañeda-Gutiérrez E, Cady RA, et al. The environmental impact of recombinant bovine somatotropin (rbST) use in dairy production. *PNAS* 2008;105:9668–9673.

Capper JL, Cady RA, Bauman DE. The environmental impact of dairy production: 1944 compared with 2007. *J Anim Sci* 2009;87:2160-2167.

Etherton TD, Bauman DE. Biology of somatotropin in growth and lactation of domestic animals. *Physiol Rev* 1998;78:745-761.

Vicini, J, Etherton T, Kris-Etherton P, et al. Survey of retail milk composition as affected by label claims regarding farm-management practices. *J Am Diet Assoc* 2008;108:1198-1203.