

**REPORT OF THE EXPERTS SCIENTIFIC
WORKSHOP ON POTENTIAL HUMAN HEALTH
RISKS FROM EXPOSURE TO FECAL
CONTAMINATION FROM AVIAN AND OTHER
WILDLIFE SOURCES IN RECREATIONAL
WATERS**

REPORT

December 21, 2011

Emory Conference Center

Atlanta, Georgia

November 15–17, 2011

U.S. Environmental Protection Agency

Office of Water

Office of Research and Development

OVERVIEW

The U.S. Environmental Protection Agency (the EPA or Agency) convened an experts workshop in fall 2011 to evaluate the state-of-knowledge on, including tools and data required for evaluating, the risks avian and wildlife fecal pollution pose to human health in U.S. recreational (ambient) waters. In addition to data gaps, the experts identified several major areas for future research that are described in the sections and appendices that follow. In general, data describing avian and wildlife pathogen occurrence, abundance, and corresponding human health risks are scarce and difficult to generalize. Further, few currently available tools for identifying and quantifying fecal inputs from avian and wildlife sources are fully developed and validated. Despite these and other data gaps, quantitative microbial risk assessment (QMRA) has been successfully applied to estimate risks associated with zoonotic pathogens in recreational water settings. Several opportunities for collaboration in the scientific and regulatory communities were identified, including cross-laboratory collaboration in the development and validation of assays and markers; development of centralized repositories of data (e.g., site-specific pathogen monitoring data); and exploration of approaches, scenarios, and case studies to evaluate the appropriateness of developing a site-specific criterion using QMRA or other approaches.

PURPOSE

This report¹ summarizes the major findings of an expert workshop convened by the EPA to identify research gaps and needs for the EPA and others to improve understanding of potential human health risks from avian and other wildlife sources of fecal pollution in recreational water settings. The workshop was also a part of the EPA's ongoing efforts to periodically convene technical experts to better inform future recreational water quality criteria development activities. Note that the objective of the workshop was not development of consensus and in many cases the workshop participants expressed diverse opinions. Twenty-six experts from the United States and Canada participated in the workshop, representing multiple U.S. federal agencies (including the EPA), state and local government, one public research agency, academia, and consultants (see Appendix A). They represented a variety of scientific disciplines including ecology, epidemiology, microbial source tracking (MST), microbiology (especially zoonotic pathogens), public health, risk assessment, veterinary medicine, and zoology. In addition to several plenary sessions to facilitate discussion of data gaps and research opportunities, all experts participated in one of three discussion tracks based on their expertise (see Text Box 1). Each discussion group had goals and initial charge questions (see Appendix B), a group lead expert, and deliberated during multiple breakout sessions over the course of the two-and-a-half day workshop (see Appendix C).

PLENARY SESSION PRESENTATIONS

Technical presentations in the first plenary session described risks associated with avian, wildlife, and agricultural sources of fecal contamination and the role of QMRA.² Nicholas Ashbolt (EPA) presented results of a QMRA of recreation in waterfowl-impacted waters; Jeffrey Soller (Soller

¹ Although this report has been reviewed in accordance with EPA policy, it summarizes the views of the individual workgroup experts—particularly the three track/group leads—and does not constitute or imply EPA policy. Further, mention of trade names or commercial products does not constitute endorsement or recommendation for use.

² QMRA applies risk assessment principles to estimate exposure to, and resulting adverse health effects from, infectious organisms.

Text Box 1: Discussion Tracks and Goals**Track 1: State-of-the-science on avian wildlife and other wildlife fecal contamination as potential sources of human pathogens***Goals*

Explore what is known about zoonotic pathogens that originate from avian and other wildlife feces and that potentially occur in recreational water. This exploration includes the relative importance of animal reservoirs, overlap between animal and human species/strains, prevalence of infection (within herd, among herds, host ranges), and abundance of zoonotic pathogens in feces.

Track 2: Human health risks from exposure to waters contaminated by feces of avian and other wildlife*Goals*

Identify the tools currently used and potentially useful for assessing wildlife and avian fecal impacts and risks for oral exposure to zoonotic pathogens during recreation. Assess the tools and identify data gaps that, if filled, could result in improved characterization of the risks.

Track 3: Avian and wildlife fecal source tracking assay development, evaluation, and validation*Goals*

Identify the various source tracking assays currently available for avian wildlife and other wildlife hosts and assess the status of these assays regarding the level to which they have been evaluated and/or validated. Discuss the role of fecal source tracking assays could have in risk assessment analyses and future water quality monitoring.

Environmental) presented results from a QMRA of recreation in livestock- and poultry-impacted waters; while John Ravenscroft (EPA) explored the role of QMRA and epidemiology studies within the regulatory framework. Both QMRA studies estimated the potential human health risks of gastrointestinal illness [GI] associated with a specific density of fecal indicator bacteria but using different sets of reference pathogens and exposure routes. Impacts from waterfowl were characterized from the direct deposition of bird feces, whereas livestock and poultry impacts were characterized from land application of fecal material with subsequent rain event mobilization of pathogens and fecal indicator bacteria. In the waterfowl QMRA, *Campylobacter* accounted for the majority of risk while *Salmonella* (the only other reference pathogen) accounted for a relatively small part of the risk. In the livestock and poultry study, which included a similar exposure scenario to that used in the waterfowl QMRA, *Salmonella*, *Campylobacter*, *Escherichia coli* O157, *Cryptosporidium*, and *Giardia* served as reference pathogens. The reference pathogens accounting for the majority of risk differed for cattle, swine, and poultry wastes, with multiple reference pathogens contributing significant risks from cattle feces in recreational waters.

Discussion and questions arising from the QMRA presentations included model selection and validation. Several experts noted that some direct source of pathogens (swimmers) and indirect sources (e.g., pathogens originating from human sources but amplified in aquatic environments such as by shellfish) were not included in the QMRA models. The presenters noted that although the QMRA framework is amenable to including additional fecal sources, data for characterizing such alternative sources are limited, and that the alternative fecal sources are heterogeneously distributed among recreation sites.

Michael Beach of the U.S. Centers for Disease Control and Prevention (CDC) gave a presentation on waterborne zoonotic pathogen disease transmission, outbreaks, and surveillance in U.S. recreational waters. Dr. Beach emphasized the uncertainty associated with attribution of illness among settings (e.g., food exposure versus drinking water exposure versus recreational exposure).

Because CDC is currently re-evaluating methods for attributing illness among settings/sources, estimates of illness rates associated with recreation in surface waters might increase in the future. Dr. Beach also emphasized the association of non-GI endpoints with avian and other wildlife sources of fecal pollution, such as swimmer's itch and respiratory infections.

Questions to Dr. Beach related largely to illness attribution and risks posed by viruses in recreational water settings. Most documented recreational water outbreaks in recent years have occurred in treated swimming pool settings, although it is generally recognized that many outbreaks associated with recreation in untreated (natural/ambient) waters go unreported. Notably, to date, no ocean outbreaks and only one Great Lakes recreational water outbreak have been reported. Generally, viruses from avian and wildlife sources are expected to pose limited risk to humans because they are usually host-specific.

DISCUSSION GROUP FINDINGS

DISCUSSION TRACK 1

The experts in discussion track 1 were charged with identifying important fecal-associated zoonotic pathogens and avian and wildlife host species associated with U.S. recreational waters, and assessing the current ability to detect and distinguish between the pathogens. These discussions were organized by pathogen grouping (viruses, bacteria, protozoa, and others) and their occurrence in avian and/or warm-blooded, non-avian wildlife. The findings were compiled into a summary table representing the experts' current understanding of the significant hosts, risk factors, and documented outbreaks associated with each pathogen or group of related pathogens. The table is presented in its entirety in Appendix D and key results are summarized below.

Detection and identification of many microbial pathogens in aquatic environments is made difficult by the complex, uncertain, and sometimes still evolving taxonomy of the pathogens (e.g., microsporidia are now classified as fungi), and because host-specific markers for most of the zoonotic pathogens identified by the discussion track 1 experts (see Appendix D) have yet to be developed. These factors are particularly important for *Campylobacter/Arcobacter*, for which new species continue to be identified, and because virulence in humans among these species is expected to vary widely.

Among bacterial zoonotic pathogens, *Salmonella* and Shiga toxin-producing *E. coli* (STEC; especially *E. coli* O157) were assessed by the group to have low prevalence among birds and other wildlife pollution sources (with the possible exception of feral pigs), whereas *Campylobacter* and *Leptospira* were assessed to be more prevalent. *Leptospira* was noted to be of particular public health concern because of its known implication in waterborne disease outbreaks, prevalence in multiple wildlife species, and its unusual transmission route (i.e., only excreted in urine of hosts but can be transmitted by oral ingestion or dermal contact). At present, *Leptospira* has not been included as a reference pathogen in any of the EPA's QMRAs because studies specific to wildlife risks have yet to be conducted (e.g., distribution of *Leptospira* serovars in wildlife). Important avian and wildlife host species include birds, deer, and rodents (e.g., seasonal migrations of sandhill cranes as sources of *Campylobacter*).

The group emphasized the public health importance of protozoan pathogens *Cryptosporidium*, *Giardia*, and *Toxoplasma* because of the high probability that exposure to low numbers of organisms can initiate infection (*Cryptosporidium* and *Giardia*), their stability in the environment, and their direct connection with documented waterborne disease outbreaks in the United States and

abroad. *Cryptosporidium* has been shown to survive for extended periods in aquatic environments. Waterborne outbreaks of cryptosporidiosis have been attributed to wildlife sources, although the distribution of human-infectious species of *Cryptosporidium* among wildlife species is not fully established. *Giardia* is also an important waterborne zoonotic parasite, particularly given its well-known occurrence in beavers that often reside in or upstream of recreational waters. As with *Cryptosporidium*, *Giardia* species/strains/types can be associated with multiple host species, and current microscopy and phenotypic methods for their detection might not be sufficient for identifying populations that pose specific human health risks. *Toxoplasma* is believed to be prevalent in human and some animal populations (especially feral cats), although the range of the animal hosts and their access to recreational waters might be limited. It has not been linked to a waterborne disease (toxoplasmosis) outbreak in U.S. recreational waters.

While there are many bird and animal viruses with zoonotic potential and that may merit future regulatory attention, the group agreed that their potential risks are, at present, not as high as those of bacterial and protozoan pathogens in the United States. Factors that raise concerns with respect to viruses include the potential for recombination (generation of human-infectious viruses), likely current under-reporting of virus incidence in environmental samples due to poor recovery rates of current detection methods, and a clear role in dissemination of highly-pathogenic avian influenza (HPAI) H5N1 in wild bird populations. The group emphasized the importance of host species range issues and seasonality/migrations for host species for zoonotic pathogens that might occur in U.S. recreational waters. For example, the prevalence of HPAI among wild birds is generally increased during autumn and early winter months.

DISCUSSION TRACK 2

Discussion track 2 experts were charged with assessing the tools and data required for evaluating human health risks associated with recreation in U.S. waters with avian and wildlife fecal pollution sources. The group developed two detailed recreational water scenarios to ground their deliberations, help identify data and data evaluation needs, and suggest appropriate uses of these types of risk evaluations.

Scenario 1

The first scenario was chosen to depict the most basic conditions under which the development of a site-specific criterion might be considered. It involves the identification and use of tools and processes at a relatively remote site that a sanitary survey (characterization) has shown to be predominantly impacted by waterfowl (and neglecting swimmer loads, which are assumed to be small). As part of this scenario, microbial water quality monitoring results for enterococci (fecal indicator bacteria) are 45 CFU/100 mL as a geometric mean (season average), indicating that the water body is not attaining its designated use as recreational water. Because the local officials believe that the enterococci seasonal average is not indicative of human health risk, development of a site-specific criterion is considered. A site-specific criterion might avoid the need to develop a total maximum daily load (TMDL) that may be costly and provide no practical beach management options, and maintain public access to the beach.

Developing a site-specific criterion in this case would begin with a comprehensive sanitary survey identifying potential fecal pollution sources. Assuming an epidemiology study is not cost effective (and is expected to have low statistical power given the anticipated low incidence of GI in swimmers) for such a relatively remote beach, QMRA could be used to identify the site-specific

water quality criterion (fecal indicator bacteria densities or other measures such as waterfowl counts) consistent with a benchmark level of risk. In this scenario, the QMRA would assume risks arise from direct deposition of feces into the receiving water by waterfowl (i.e., waterfowl are the primary source of pathogens and indicators at the recreation site) and, as a conservative estimate, that 100 percent of *Campylobacter* from the waterfowl are human-infectious. Several important issues will need to be considered regarding the use of QMRA in this scenario, such as collection and incorporation of robust data on pathogen and indicator occurrence, selection of an appropriate set of reference pathogens, and association of risks with the indicator used in the criterion. Other considerations include characterizing site-specific features such as hydrodynamics, other sources of spatial and temporal variability, export of pathogens and indicators from sands and sediments, and other biological and physical processes that can impact the occurrence of pathogens and indicators in the recreational water. To date, QMRA models of animal (avian and livestock/poultry) impacts on recreational waters have used literature-based estimates of pathogen prevalence and abundance in feces, and of indicator bacteria abundance in feces. For the most part, the group agreed that the latter approach for developing source terms for the QMRA, while valid for exploring general risks associated with animal fecal sources, may not be sufficient for establishing a site-specific criterion for a scenario 1 beach. Fecal pollution dynamics in the occurrence and abundance of pathogens in wildlife and birds are widely variable among sites and characterizing these differences based on literature-reported ranges might result in significant over- or underestimation of risk. Further, reported ranges of pathogen occurrence in the United States are scarce and anecdotal.

In light of the low densities of pathogens likely present at a scenario 1 beach, scat/guano (feces) monitoring was assessed to be a better approach for establishing pathogen occurrence than water monitoring. Pathogen monitoring schemes used to develop QMRA fecal source data should consider migratory patterns of waterfowl and seasonal differences in pathogen occurrence in the waterfowl, transparency and quality assurance, and the possible establishment of approved/validated analytical techniques.

A credible QMRA model based on site-specific pathogen data, fate and transport, and hydrodynamics could then be used to establish an indicator level at which risks are consistent with benchmark risks. For example, a 75th percentile geometric mean enterococci density at a specified risk level could be used to develop a site-specific criterion for a scenario 1 beach. The discussion group noted that use of indicators other than fecal indicator bacteria densities (e.g., waterfowl counts) might warrant consideration as a means for expressing a site-specific criterion.

Scenario 2

The second, more complex scenario evaluated by discussion group 2 entails site evaluation and development of a site-specific criterion for a Lake Erie beach impacted primarily by waterfowl feces, but that also has potential human fecal sources (e.g., a visitor center with a small septic system and regular visitors). As in scenario 1, the enterococci density exceeds the current water quality standard and local officials seek to explore a site-specific criterion because the risks associated with recreation at the beach are expected to be lower than those associated with sites impacted primarily by human fecal sources (e.g., treated sewage). In contrast to scenario 1, there is uncertainty regarding the relative contribution of human and non-human fecal sources.

As with the scenario 1, a comprehensive sanitary survey is a first step in evaluating a site-specific criterion in scenario 2. Because there are multiple fecal sources, an estimation of the relative contribution of human and non-human fecal pollution sources to indicator organism measurements

is needed. One potential approach has been recently demonstrated by Wang et al. (2010).³ Their technique entails the use of universal and host-specific *Bacteroidales* markers and statistical inference to develop an estimate of the contribution of specific fecal pollution sources and the uncertainty associated with those estimates. The discussion group noted that although this technique is promising, it is relatively new, and a pathway for using the technique to establish a site-specific criterion (e.g., in terms of enterococci density) is not yet established.

The scenario 1 and 2 discussions lead to the development of a general model, illustrated in Figure 1, of the process by which a site-specific criterion might be developed. Note that if the suspected fecal source is not avian and/or wildlife then the flowchart may need to be modified. Jurisdictions will be unlikely to initiate the relatively costly process of developing a site-specific criterion if sites are already attaining their designated use(s). Development of a site-specific criterion entails determining the likely fecal sources, quantifying the contributions from the sources, collecting pathogen data specific to the site (or specific to groups of similar sites), and using QMRA or another tool. Confirmatory water quality monitoring was suggested by the group because initial

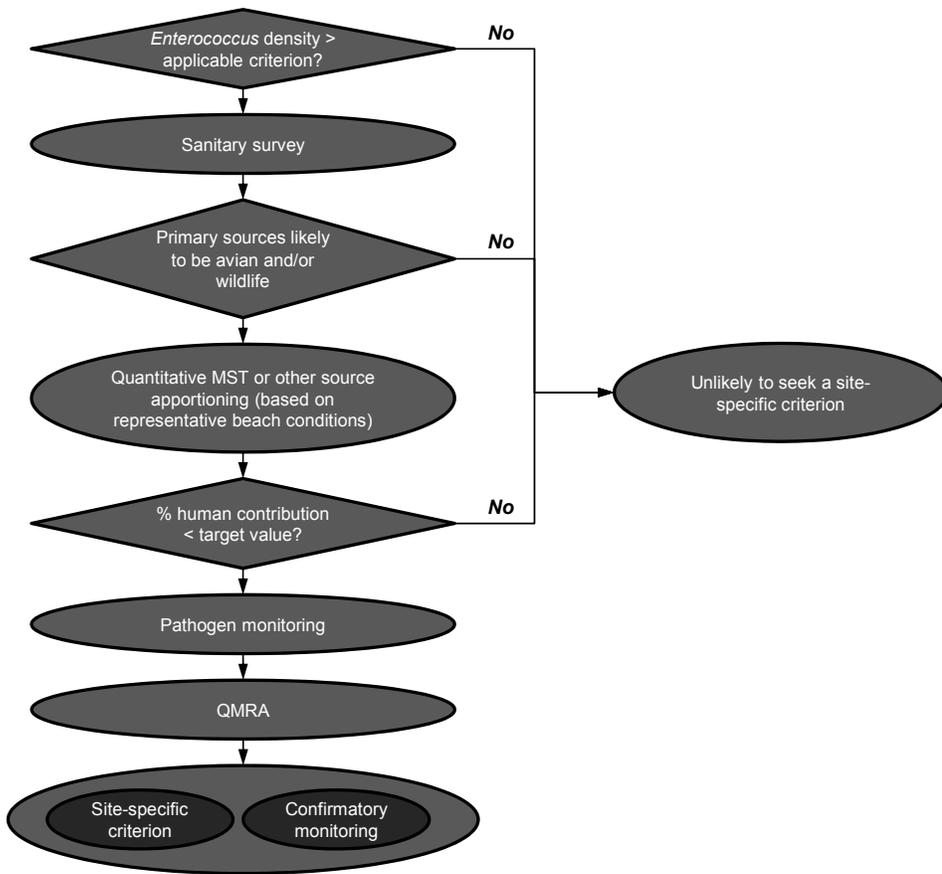


Figure 1. Flow diagram of potential steps toward a site-specific criterion for recreational sites where birds and/or wildlife are suspected to be the primary fecal source.

³ Wang, D., Silkie, S.S. Nelson, K.L., and Wuertz, S. 2010. Estimating true human and animal host source contribution in quantitative microbial source tracking using the Monte Carlo method. *Water Research* 44(16): 4760–4775.

monitoring efforts are of limited duration and fecal sources and their characteristics will change over time.

Any Scenario

Broader topics raised by the group during discussion of the two scenarios, and those not specifically associated with either scenario, include the following:

- Tools identified for assessing human health risks associated with avian and wildlife sources include epidemiology studies, QMRA, sanitary surveys, MST (also called fecal source tracking or FST), TMDLs, scat/guano surveys, and local public health surveillance (e.g., recreational waterborne disease outbreaks).
 - Costs, benefits, and disadvantages of these tools for beach management by local public health authorities.
 - Importance of sanitary surveys in evaluation of health risks at specific recreational sites or groups of related sites.
- The direct exposure route (direct deposition of avian or wildlife feces into recreational waters) is considered to be a worst-case scenario, except when fate and transport property differences among pathogens and indicators alter ratios of pathogens and indicators between fecal source material and recreational waters.
- Data requirements underlying credible risk estimation (using literature-based data alone may be insufficient).
- Environmental sampling strategies for developing robust data for risk evaluation.
- Role of different health endpoints (beyond GI outcomes) and severity in evaluation of site-specific risks and the association of such endpoints with traditional fecal indicator bacteria measurements.
- For most waterborne pathogens of public health relevance, there are sufficient data for their evaluation in risk analyses.
 - Notable exceptions include influenza, HPAI H5N1, mycobacterium avium complex (MAC), *Chlamydophila psittaci*, microsporidia, avian schistosomes, Hepatitis E virus, *Leptospira*, *Toxoplasma*, and fungi and helminthes.

The group also discussed the appropriate selection of indicators and their relationship to risks. This topic generated broad debate within the discussion group. Some participants noted the advantages of indicators other than fecal indicators, including more direct connection to fecal pollution source, potentially easier measurement of the indicator (e.g., waterfowl counts), and reduction in the impact of confounding factors such as environmental (re)growth of fecal indicator bacteria. Other experts expressed concern over the integration of indicators other than fecal indicator bacteria with numeric criteria that are currently based exclusively on fecal indicator bacteria counts. There was also concern that detection of enterococci, which have been shown to survive and even propagate in the aquatic environment (including sediments and beach sand), might not be indicative of recent fecal contamination. Enterococci and *E. coli* have also been shown to concentrate and grow within the algal mats along the shores of the Great Lakes where birds feed on the invertebrates, scavenge food, and defecate.

DISCUSSION TRACK 3

The experts of discussion track 3 were charged to summarize the availability, performance, and use of MST assays and methods for investigating recreational water quality. The group elected to

exclude vectors and domestic (companion) animals from discussions, but to include warm- and cold-blooded animals—although the latter did not receive as much attention as little MST research has been published for these animals. Consideration was also given to secondary habitats such as *Cladophora* (green algae) and beach sands. The discussion group first noted that the answer to their overall goals and initial charge questions (see Appendix B) depends upon the intended use of the MST assay or analysis, which could include MST as components of beach management and water quality monitoring, TMDL development, best management practice (BMP) assessment, and epidemiology studies.

Discussions began with an exploration of the differences between evaluation and validation of MST methods. Validation was described as a more rigorous process than evaluation and implies demonstration of the method under environmental conditions. It also implies some degree of field study or application of the method. Evaluation entails determining whether markers, assays, and methods are effective. Considerations that the group identified as useful for distinguishing evaluation and validation of MST methods are presented in Table 1.

The group also identified both avian-specific markers and wildlife and avian pathogens described in peer-reviewed studies. These include (1) the Gull-2 marker that targets *Catellibacterium marimammalium* in gulls; (2) avian-specific (gulls, ducks, geese, and chicken) fecal 16S rDNA sequences; (3) *Bacteroides* markers for geese and ruminants; (4) host-adapted *Cryptosporidium* species for geese and rodents; and (5) viral pathogens (polyomavirus, adenovirus, and norovirus) for different host groups (humans, mammals, and avian). Notably, most of the published work to date relates to a relatively small number of avian hosts and on the level of validation achieved. However, the group found that none of the avian and wildlife markers described in the literature (see also Appendix E) are currently ready for use based on the evaluation and validation considerations described in Table 1.

Numerous obstacles to apportioning indicators among fecal pollution sources were identified. Overcoming these obstacles will be important in application of MST in TMDL development, evaluation of BMP performance, and use of MST in other applications requiring source apportionment. A significant obstacle to use of markers for estimating partitioning of fecal sources was identified as the environmental variability of the markers. Marker compositions can vary among individuals of the same host species, historically within populations at a specific site, with

Table 1. Evaluation and validation considerations for methods

Evaluation	Validation
<ul style="list-style-type: none"> • Number of samples, hosts, replicates, etc. (might be unknown) <ul style="list-style-type: none"> ○ Host-specificity ○ Stability ○ Genetic ○ Geographic ○ Temporal • Marker abundance • Marker distribution • Performance metrics <ul style="list-style-type: none"> ○ Percent occurrence ○ Sensitivity ○ Limits of detection 	<ul style="list-style-type: none"> • Fate and transport properties <ul style="list-style-type: none"> ○ Growth ○ Survival • Relationship to <ul style="list-style-type: none"> ○ Source ○ Pathogens of interest ○ Regulatory indicators ○ Health risks ○ Amplification in the environment

host diet, in abundance among individuals, and with other factors. It is important to consider this variability when sampling plans are developed and that may necessitate the use of multiple markers as part of a robust MST effort. Confidence in markers can be increased through

- Cross-laboratory collaborations (standardization of protocols and efforts to demonstrate reproducibility).
- Modeling and statistical analyses.
- Use of multiple markers for the same target.
- Standardization of sampling (e.g., through issuance of a guidance).
- Use of historical site knowledge (e.g., land use data).

Basic research needs identified by the group were generally related to the impact of environmental conditions on marker detection and abundance. Use of markers in MST for fecal source apportionment will require an understanding of the biology and ecology of the marker within hosts and outside host species. Within-host factors that could be explored include seasonality, host range, fecal shedding rate, abundance of marker/feces composition, influence of diet on fecal shedding, and variation in marker presence and abundance with animal health and immunological status. Factors in environmental settings that could benefit from exploration include the (re)growth survival, and environmental reservoirs of markers; transport properties of markers (e.g., partitioning to soils and riparian vegetation); and potential for mutation of target genes. General information describing the impacts of the intra- and extra-host environments on markers might be derived from studies on the genetic basis of host specificity and the genetic basis for environmental persistence.

The discussion group identified each step in the analytical processes for detecting/quantifying markers as areas for research. Specific items identified include understanding the impact of sample handling and collection on results, matrix effects, inhibition, sensitivity, and specificity. Elements of analysis not strictly related to methods, but still requiring development and research, include standardization of protocols, creation of a centralized source of standard materials, standardization of unit(s) of measure, and specification of acceptable error rates (e.g., for replicate measurements or standard curves).

Elements of sampling requiring improved definition or research include development of techniques for determining the number and locations of sample collections (including the minimum number of geographic locations required), development of techniques for quantifying the uncertainty and variability associated with measurements, and determining the number of target- and non-target samples needed for performance criteria. Similar to analytical methods, some non-technical elements of sampling also require development, including improved data management capabilities and techniques for handling non-detects.

The discussion group envisioned MST as an element in a tool box of techniques for integrative water quality monitoring. Tools that could accompany MST include the following:

- Databases of technical publications.
- Online training for staff and scientists conducting sampling.
- Centralized sources of protocols and guidance for method, sanitary surveys, and QMRA data needs.
- Case studies.
- Data sources such as Natural Resources Conservation Service soil and habitat spatial data.

- Models used for predicting avian and wildlife population sizes and migratory patterns, geographic data for infrastructure (e.g., sewer networks, stormwater outfalls, lift stations, urbanized areas).

SUMMARY OF MAJOR DATA GAPS AND RESEARCH NEEDS

All three discussion group were tasked to identify data gaps and research needs specific to their initial charge questions and additional research questions were identified by the experts in plenary sessions. Although the summaries provided below emphasize major points of discussion, many other data gaps and research opportunities were identified by workshop experts (see Appendix F).

Generally, pathogen and indicator data for avian and wildlife hosts are sparse. This lack of data limits efforts to link fecal sources with health risks and estimation of pathogen loads associated with specific host species. A key element needed to improve the association of health risks with avian and wildlife sources in U.S. recreational waters is a **detailed understanding of the ecology of key pathogens in significant host species**. This would allow prioritization of zoonotic pathogens of public health importance and support an improved and reliable interpretation of MST marker occurrences and abundances. Studies of the ecology of pathogens and their surrogate markers could include quantification of their fate, transport, and survival in the environment, as well as their abundance and prevalence in specific host species.

A major data gap is **the extent to which host-adapted pathogens are able to infect humans**. Addressing this data gap will require collecting additional data on human infection from a wide array of pathogen species/strains/types (which are likely to be derived from animal studies, outbreak analyses, or modeling) and on pathogen carriage among diverse wildlife and avian hosts, or development of genomic and virulence markers whose detection is indicative of a likelihood of the presence of waterborne pathogens that can infect humans. Improved understanding of the ability of zoonotic pathogens to infect humans would benefit QMRA analyses through generation of improved dose-response models and selection of appropriate reference pathogens.

Uncertainty exists regarding **the appropriateness of current reference pathogens to represent zoonotic pathogens of public health importance**. Pathogens beyond the currently used reference pathogens, and which have been primarily developed for livestock/poultry and waterfowl fecal sources, are generally associated with data gaps—including the hosts in which they typically occur, fecal abundances and prevalence rates for different host species, and dose-response characteristics. Pathogens identified as potentially important additions to the reference pathogens for wildlife and avian impact QMRAs include MAC, Hepatitis E virus genogroup 3, *Leptospira*, and HPAI viruses.

Improved understanding of human responses to pathogen species/strains/types from animals would support improved QMRA modeling and potentially narrow the ranges of host species and pathogens for which research is warranted. Research activities targeting improved understanding of human response to avian- and wildlife-origin pathogens include connecting genomics to virulence and host specificity (toward better monitoring tools), studying virulence differences in laboratory cultures used in feeding studies and environmental pathogen populations, evaluating the applicability of dose-response models based on outbreak data to analysis of risks in recreational waters, and determining the applicability of current dose-response models to additional populations and strains than those used in published feeding studies.

Method development research priorities include basic analytical method advancements such as testing the reliability of assays, standardization, and increased collaboration among researchers and

laboratories regarding reference strains and generation of databases of metadata associated with MST analyses. Marker development for relevant fecal sources and linked to specific sites is a priority. Specific hosts of interest include pan-bird populations, deer, multiple rodents (especially voles, muskrats, beavers, and raccoons/opossum/skunks), feral cats, fur seals, and manatee. Alternative markers such as pathogenic organisms (e.g., host-specific viruses) could also be developed and evaluated. Techniques for apportioning sources via MST analyses were also identified as a research need. One such probabilistic approach is available for at least the *Bacteroidales* MST method (Wang et al. 2010; see footnote 3), but would still require the development of additional avian and wildlife-associated genetic markers. These techniques could be used in conjunction with modeling to estimate the predominant source of fecal contamination in a water body.