Appendix A: Participants List

Experts Scientific Workshop on Potential Human Health Risks from Exposure to Fecal Contamination from Avian & Other Wildlife Sources in Recreational Waters

Expert Participants

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Robert Atwill *(Track 1 Lead)*
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Other Attendees

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Appendix B:
Charge Questions

Experts Scientific Workshop on Potential Human Health Risks from Exposure to Fecal Contamination from Avian & Other Wildlife Sources in Recreational Waters

Charge Questions

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/flock, among herds/flocks, host ranges), and abundance of zoonotic pathogens in feces.

Potential points for discussion

- What microorganisms are known to occur in avian wildlife and other wildlife feces that have the potential to be pathogenic for humans and transmissible by water during recreational water exposure?
- How prevalent are the avian and wildlife fecal-origin zoonotic pathogens that are transmissible through water? Note that prevalence may relate to inter-herd/flock, intra-herd/flock, or intra-region occurrence of the pathogen.
- Which hosts of zoonotic pathogens occurring in wildlife and avian feces pose the greatest risk to humans during recreational water exposure? What are their ranges?
- What is the state of the science in the ability to distinguish between the species/strains/types of water transmissible pathogens commonly occurring in avian and wildlife hosts?
- Focusing on predominant human routes of exposure, for which wildlife and avian fecal pathogens of consequence do we have dose-response data? Outbreak data?

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.

Potential points for discussion

- What approaches could be used to assess human health risks from exposure to fecal contamination from avian and other wildlife?
- What do we know about the exposure routes (from fecal source to human exposure) and levels of human exposure needed to affect significant human health risk of GI infection from avian wildlife or other wildlife sources?
- What are the most significant data gaps that prevent us from quantifying the relative risks of different fecal pollution sources?
- For those zoonotic pathogens identified in track 1, do we have information to support either their direct evaluation or evaluation via reference pathogens in risk assessment?
- How might findings on source tracking (from discussion track 3) be used in assessment of source- (or mixed-source-) specific 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 FST assays could have in risk assessment analyses and future water quality monitoring.

Potential points for discussion

- What fecal source tracking (FST) assays currently exist for various avian wildlife and other wildlife sources?
- How have avian wildlife and other wildlife source tracking assays been useful in past field studies?
- What performance criteria would be of critical importance for future evaluations of host-specific FST assays?
- Which currently developed assays satisfy these performance criteria and are ready for use now?
- What level of evidence identifies/quantifies relative contributions of various sources?

Appendix C: Workshop Agenda

Experts Scientific Workshop on Potential Human Health Risks from Exposure to Fecal Contamination from Avian & Other Wildlife Sources in Recreational Waters

Agenda

Tuesday, November 15	
7:00–9:00	**Breakfast (on your own)**
8:00–9:00	**Packet pick up**
9:00–9:15	**Overview of Logistics** Facilitator
9:15–10:00	**EPA Welcome & Workshop Objectives** Mark Rodgers, USEPA/ORD
10:00–10:15	**Agenda Review, Workshop Format and Process** Facilitator
10:15–10:30	**Break**
10:30–11:15	**Plenary Session 1—QMRA Framework** John Ravenscroft, USEPA/OW, Nicholas Ashbolt, USEPA/ORD, and Jeffrey Soller, Soller Environmental
11:15–12:00	**Plenary Session 2—Zoonotic Outbreak Data** Michael Beach, CDC
12:00–1:30	**Lunch (on your own)**
1:30–1:45	**Review Logistics and Final Instructions to Workgroups Break**
1:45–2:00	**Break & Move into Topic Sessions**
2:00–5:30	**Topic Sessions**
5:30	**Adjourn**
6:00–9:00	**Dinner (on your own)**
7:30	**Planning Committee & Track Lead Check-in**

Wednesday, November 16

7:00–8:00	**Breakfast (on your own)**
8:00–10:00	**Work in Topic Area Teams**
10:00–10:15	**Break & Move into Plenary**
10:15–12:15	**Plenary** • Group report outs • Feedback
12:15–1:30	**Lunch**
1:30–5:30	**Individual or Combined Topic Area Team Sessions** *This time can be used for workgroups to meet separately or jointly as determined by the Track Leads.*
5:30	**Adjourn**
6:00–7:00	**Dinner (on your own)**
7:00–9:00	**Group Bowling Event (on site)**

Thursday, November 17

7:00–8:00	**Breakfast (on your own)**
8:00–10:30	**Work in Topic Area Teams**
10:30–12:30	**Topic Area Summaries**
12:30–1:00	**Closing Remarks**
1:00	**Adjourn**

Appendix D:
Track 1 Summary Table

Microorganism	Hosts/Prevalence	Dose-Response Data	Outbreak Data	Risk Factors/Concerns	Comments
Avian					
Viruses					
Influenza (H5N1)	Very low in wild birds (H5N1); low pathogenicity avian influenza 1–30% in U.S. wild birds depending on season and species; between 1–20% detection rate in water and environmental samples	In animals, high path AI infections dose (ID_{50}) 10–1000 pfu in susceptible; low path AI 10^4–10^6 pfu (oral)	Unknown in humans at present, but introduction to new areas by wild birds documented and some avian deaths reported associated with municipal water supplies	Not currently in U.S.; role for environmental contamination (fecal-oral), migratory birds; potential for widespread infection; recombination of genes is major concern for virulence emergence	WHO report on H5N1 and water supply (potential); detection specificity issues; need to improve public health awareness and be prepared; many common strains in U.S. non-pathogenic for people
Bacteria					
Campylobacter	Unknown but as a genus has wide range of prevalence; species pathogenic to humans varies widely between species, might vary geographically	Large range, varies by species; estimates available in literature	Drinking and recreational waters in Europe limited data in U.S.	Large species diversity in birds and unknown which are human pathogenic (besides *C. jejuni*); little known about species distribution in wild birds	Few systematic surveys in wild birds using modern detection methods; limited culture methods may bias animal survey results
Chlamydophila psittaci	Wild parakeets; unknown	Unknown	Unknown	Sick birds, water transmission potential	Southeast U.S., mainly coastal
Mycobacterium avium complex (MAC)	Unknown	Unknown	Unknown	--	Need to learn more; biofilm concern, possible link to inflammatory bowel disease
Salmonella	<10% in healthy birds; outbreaks more common in passerines; prevalence can be higher in sewage/manure-associated birds (mainly non-U.S.)	Large range, lower in children, varies by serotype; estimates available in literature (e.g., WHO reports)	Drinking water outbreaks (birds); limited recreational water data	Various serotypes across avian species; seasonal build-up of large local populations; outbreaks in bird feeders leading to human contact; wild birds in urban areas	Some serotypes very persistent (typhimurium); Platte River study results (temporal shifts in migratory bird populations and waterborne pathogens)
Shiga toxin-producing *E. coli* (STEC)	0–5% (O157) depending on species, California data found high in cowbirds, crows, and water birds such as herons, geese; limited data on non-O157 STECs	No data on any avian strain	Samadpour et al. 2002 (ducks suspected)	Possible higher risk of infection when forage around livestock (O157)	Methods for non-O157 under rapid development (detection issues); young birds can be colonized by many pathogens

Protozoa					
Avian schistosomes	Wild ducks and geese; varies by location and snail population	Unknown	Unknown	Life-cycle with gastropods, dermatitis (swimmer's itch)	Mostly smaller bodies of water
Cryptosporidium spp.	Large species diversity in birds; prevalence unknown in wild bird species	Human feeding trials from human origin *C. meleagridis* (80%, 10^5 by Chappell et al. 2011)	Unknown	Human pathogenic species is *C. meleagridis*	Only avian-specific *Cryptosprodium* spp. pathogenic in humans, greater public health concern outside U.S.; migratory bird/livestock concern/mechanical transport (research area)
Fungi					
Microsporidia	~10% in pigeons; unknown for other birds	No data	Unknown	*Encephalitozoon hellem Enterocytozoon bieneusi* (many genotypes, some very host-specific, others not, taxonomy concerns)	Actual number of people infected in U.S. small; *E. bieneusi* outbreak in HIV patients in France; recently moved from protozoa to fungi
Non-Avian Wildlife, Warm-Blooded					
Viruses					
Hepatitis E	Assume feral pigs given in domestic pigs; unknown for other species	Unknown	Unknown	Found in water samples possibly contaminated by wildlife, such as wild pigs	Research ongoing in China in various domestic animals
Bacteria					
Campylobacter	Widely distributed among many wildlife species; prevalence can vary widely	Large range, varies by species; estimates available in literature	Yes	Density, proximity to water sources	Taxonomy is rapidly evolving as new species are discovered
Leptospira	Rodents, skunks, raccoons, foxes; prevalence unknown	Need to assess literature, data likely available	Yes, U.S. and abroad, typically from unknown source(s) (see Narada et al. 2005 in Japan)	Exposure to urine, low infectious dose	Tropical; half of U.S. cases in Hawaii
MAC	Bears, raccoons, coyotes, and other wildlife; prevalence unknown	Unknown	Unknown	--	--

Salmonella	Widely distributed among many wildlife species, but with low (0–10%) prevalence	Large range, lower in children, varies by serotype; estimates available in literature (e.g., WHO reports)	Yes	Density, proximity to water sources	High diversity among serotypes detected; human pathogenic potential varies
STEC	Wild ruminants (e.g., deer); 0–10% (non-O157); 5% in California feral pigs (O157)	Unknown, but dose-response data from domestic ruminants available	Japan, untreated water source: feces from "wild animals"	Density, proximity to water sources	For non-O157, few systematic animal surveys, though extensive research is currently ongoing
Protozoa					
Cryptosporidium spp.	Large diversity (species and genotype) in mammals; 0–10% prevalence	Multiple feeding studies for *C. parvum*, relatively low infectious dose	Drinking water outbreak from *C. cuniculus* from rabbit(s) in U.K; recreational water outbreaks from *C. parvum*, source unknown	*C. ubiquitum*, *C. canis*, *C. parvum*, and *C. cuniculus* found in humans and a variety of wild animals	--
Giardia duodenalis	Large genotype diversity in mammals; 0–30% prevalence	Human trials, dated, 10–25 cysts	Several drinking water outbreaks, possibly linked to beavers; recreational water outbreaks, source unknown	Only assemblages A & B are human pathogens, but have been found in a variety of wildlife	Beavers have been linked to several waterborne disease outbreaks
Toxoplasma	Feral cats, wild felids; 5–15% prevalence	Different by genotype and host	Yes in North, Central, and South America	Pregnant women of high concern (danger to fetus) but can be pathogenic to overall population	Catch/neuter/release programs controversial in coastal communities; marine mammal impacts proves land-marine hydrologic connections; contaminated shellfish

Fungi					
Microsporidia	Found in a variety of mammals; prevalence unknown	No data	Unknown	*Encephalitozoon hellem* *E. intestinalis* *E. cuniculi* *Enterocytozoon bieneusi* (many genotypes, some very host-specific, others not, taxonomy concerns); immune-compromised at most risk	Actual number of people infected in U.S. small; have been detected in U.S. waters
Helminths					
Baylisascaris procyonis	Raccoons, 10–30% prevalence	Unknown	Unknown	Density, proximity to water sources	Not much known

Appendix E:
Track 3 Summary Table

Published Methods

Marker	Target Host(s)	Target Organism	Reference	Known Source Validation	Field Study Validation
16S rRNA (Gull- 2)	Gull	*Catellicoccus marimammalium*	Lu et al. 2008		
Avian-specific 16S rDNA (GFB GFC GFD)	Gulls, geese, ducks, and chicken	Varied, including *Fusobacterium*, *Catellicoccus*, and *Helicobacter*	Green et al. 2011		
16S rRNA (CGOF1-*Bac* CGOF2-*Bac)*	Geese	*Bacteroides*	Fremaux et al. 2010		
16S rRNA (E2)	Duck	*Desulfovibrio*	Devane et al. 2007		
16s rDNA (CF128, CF193, BacR)	Ruminant	*Bacteroides* and *Prevotella*	Bernhardt and Field 2000		
Microchondrial (mt) DNA	Human, bovine, ovine, porcine, and chicken	Human, bovine, ovine, porcine, and chicken	Kortbaoui et al. 2009		
Cryptosporidium DNA	Rodents	*Cryptosporidium*	Lu et al. 2009	Yes	No
Cryptosporidum DNA	Geese	*Cryptosporidium*	Zhou et al. 2004		
Polyomavirus DNA	General avian, geese, and mammal strains	Avian polyomavirus and goose hemorrhagic polyomavirus	Perez-Losada et al. 2006		
Viral pathogens and bacteriophage DNAs and RNAs	Human, pigs, and ruminants	Adenovirus, norovirus and F+ RNA bacteriophage	Wolf et al. 2010		

Appendix F: Preliminary Data Gaps & Research Needs/Opportunities

Preliminary Data Gaps and Research Needs/Opportunities

Group Research Needs

(identified during final plenary session— November 17, 2011)

1) Prioritizing pathogens
2) Testing reliability of assays
3) Linking pathogen – indicator – host (both loadings and occurrence)
4) Development of good additional markers for relevant fecal sources
5) Pathogen data for modeling – connected to sources, health risks
6) Determine which host species are in high abundance and generate high loads; must also know pathogen abundance distribution and # of pathogens that are human-infectious
7) Prioritize wildlife species for marker development. Could be development of a decision-tree approach in which both loadings and health risks are incorporated. What attributes would we use for prioritizing? Specific species identified for consideration include
    a) Deer
    b) Rodents (voles in specific)
    c) Muskrat
    d) Raccoon/opossum/skunk
    e) Pan-bird markers
    f) Beavers
    g) Fur seals
    h) Manatee
    i) Feral cats
    j) Sea otters/sea lions/sea elephants
    k) Dolphins
    l) Mountain lions
    m) Mongoose
    n) Rabbits
    o) Swine
    p) Wild boar
8) Linking markers to specific sites
9) An alternative to markers is host-specific pathogens – particularly host-specific viruses
10) Development of scenarios for use in generating relevant/important gaps
    a) Scenario 1 – Bird Site
        i) Literature-based vs. site data collection – consensus in track 2 is that scat/guano sampling is critical
        ii) What weight of evidence supports a “no human impact” assertion?
11) Techniques for apportioning among sources based on MST
12) Determine the real extent of waterborne exposure
13) Need for standardization/collaboration for reference strains; also for a database of metadata associated with the occurrence of markers

14) Connecting genomics to virulence and host specificity (toward better monitoring tools)
15) Better dose-response knowledge
    a) Are dose-response models based on outbreak data relevant to recreational waters?
    b) What is the applicability of current dose-response models to other populations and strains other than those used in feeding studies?
16) Studying the difference in virulence of lab cultures used in feeding studies and virulence of environmental pathogen populations
17) Inexpensive methods to process recreational water (e.g., samples collected during storms); could be a major technological hurdle

Track 1

Data Gaps

General

- Across many of these pathogens there is a need for the identification of strains/isolates/species that are pathogenic to humans
- Lack of documented U.S. recreational water outbreaks for zoonotic pathogens from birds and wildlife *[overlap with # 12 above?]*
    - Need for a uniform approach for assessing exposure routes for disease outbreaks and pathogenic-specific case control studies; need to overcome existing shortcomings and biases for attribution (e.g., bias toward foodborne outbreaks and drinking water vs. recreational water)
    - Need for methods development to detect bacteria and viruses from recreational waters; matrix effects and culture effects *[overlap with # 2 above?]*
- Better understanding of general prevalence and geographical distribution of zoonotic pathogens in targeted wildlife species *[overlap with # 6 above?]*
    - E.g., pathogenic *Campylobacter* and *Salmonella* in Canada geese
    - E.g., human pathogenic STEC (Shiga toxin-producing *E. coli*) in deer and feral pigs
- Pathogen load and duration of shedding needs to be better understood at a population level for key zoonoses and birds and wildlife *[overlap with # 3 or 6 above?]*
- Unknown correlation between current QMRA reference pathogens to other zoonotic pathogens

Bacteria

- Prevalence of human pathogenic strains/isolates/species of *Salmonella*, *Campylobacter*, and STEC in birds; of these *Campylobacter* is the most important *[overlap with # 6 above?]*
- Detection, quantification, and attribution of *Campylobacter* in avian populations *[overlap with # 6 above?]*
- Distribution of *Leptospira* serovars in wildlife populations *[overlap with # 6 above?]*
- Distribution of non-O157 human pathogenic STEC in wildlife populations *[overlap with # 6 above?]*

Protozoa

- Need for large scale surveys of *Cryptosporidium* and *Giardia* species and genotypes in key wildlife populations *[overlap with # 6 above?]*
- Need improved source attribution when *Cryptosporidium* is detected in a water sample – possibilities of multiple contribution species *[overlap with # 9 above?]*

Viruses

- Unknown prevalence of Hepatitis E virus and their genotypes in U.S. wildlife; need to define the role of Hepatitis E virus in waterborne disease
- NOAA has announced that influenza in a large scale seal mortality event in northeast U.S. may be a public health threat, the role of these emerging strains of influenza is unknown
- Influenza
- H5N1 (plausible environmental pathway)

Other Notes

- MAC (mycobacterium avian complex)
- *Chlamydia*
- Microsporidia
- Avian schistosomiasis
- Hepatitis E virus
- *Leptospira* – considering other exposure routes
- *Toxoplasma* (sea otters, dolphins, sea lions, sea elephants(?), feral cats, mountain lions)
- *Ascaris* or other helminths (may have dose-response models) (may be of limited relevance) (racoon, species-specific – range of helminths is unknown but there is no evidence of waterborne transmission)

Organisms that do not match reference pathogens

- *Leptospira*
- Hepatitis E virus genogroup 3 (deer, mongoose, rabbit, swine, wild boar)
- Influenza

Track 2

- Markers *[overlap with # 8 above?]*
    - Validate for different ecosystems and regions of the country
- Specific avian and wildlife species for which we need pathogen occurrence, abundance data (based on literature review [including grey literature] and sampling) *[overlap with # 7 above?]*
    - Brown pelicans
    - White-tailed deer
    - Wood storks
    - Wood chucks
    - Muskrats

    - Species in high abundance, creating high fecal load, located near water, and associated with pathogens that are highly infectious
- More information on prevalence/abundance and geographic/temporal distribution (etc.) of pathogen (we should be okay for shorebirds, livestock)
- Likelihood that strains are infectious to humans *[overlap with # 15 above?]*
- Have we got the right reference pathogens?
- Proportion of animal pathogens that is human-infectious?
    - Involves uncertainty in dose-response (strains, types), bias due to age and health condition of participants in studies, and distribution of pathogenic strains in hosts and their shedding; intestinal biota and population dynamics
- Are reference pathogens reflecting all emerging and unmeasured pathogens (e.g., avian Hepatitis E virus)?
- Transfer and amplification of pathogens via intermediate hosts
- Gaps in readiness to inform individuals who will apply for site-specific criteria
- Discussed data bases on prevalence of pathogens in scat and guano AND mobilization plus fate and transport
- What pathogen strains are found and which hosts would harbor them?

Track 3

- What animal sources should we look at and why? Prioritize top 5 avian and wildlife sources (hosts) *[overlap with # 7 above?]*
- What is the intended use of the technology we are trying to develop?
    - Clear definition of intended use of developed methods (need assessment)
- Reliability and relevance *[overlap with # 2 above?]*

Basic/Background Science

1. Correlation of pathogens and MST markers in the host and environment
2. What are the commensal organisms in the hosts of interest at the host population level?
3. Understand the ecology and fecal shedding rate of the host animals *[overlap with # 8 above?]*
    a. Seasonality
    b. Range and habitat
4. Feasibility of linking MST measurements to treatment and management actions
5. Relationship between the diet of the host animals and fecal shedding
6. Relationship between the marker of choice and the micro-biota as it pertains to immunological status (linked to # 1 above)
7. Need to know the biology of the host organism as it relates to MST
8. How do fate, transport, and survival of the markers correlate to pathogens and fecal indicators?
    a. Fate – Growth, regrowth, survival, reservoirs
    b. Transport – hydrological parameters, partitioning
    c. Fate and transport during extreme weather events

9. Distribution and relative abundance of the genetic marker within and between target and non-target hosts, including geographic distribution
10. How does horizontal transfer and mutation of the targeted gene affect the validity of an assay?
11. Identify most important ecological factors that affect fate and transport
    a. Genetic basis of host specificity
    b. Genetic basis of survivability

Analytical

1. How does sample collection and handling affect results?
2. Sensitivity
3. Specificity
4. Limit of detection; limit of quantification
5. Inhibition
6. Matrix effects
7. Reproducibility
8. Extraction procedures and efficiencies
9. Standardization of protocols: QA/QC
10. Centralized source of standard materials
11. Influence of different instrumentation and chemistries
12. Research into new detection platforms
13. Standardization of unit(s) of measure
14. Acceptable error rates (e.g., replicate measurements, standard curve)

Statistical/Sampling Effort

1. Number of target and non-target samples needed for performance criteria
    a. Keeping in mind that there are different types of fecal material for various hosts
2. Minimum number of geographical regions that should be covered
3. Centralized database *[overlap with # 13 above?]*
4. Number and location of environmental samples for statistical confidence
5. Ground truthing
6. Estimating variability and uncertainty in measurements

Integrative Water Quality Monitoring

1. Integration of GIS and hydrology data *[overlap with # 5 above?]*
    a. Relationship of MST markers to land use
        i. Sanitary infrastructure
        ii. Population density
        iii. Health risk data
        iv. Socio-economic status measures
2. What is FST/MST marker value in context of a ""tool box" approach?