

Laboratory for Infectious Disease and the Environment (LIDE)



L · I · D · E

LABORATORY FOR INFECTIOUS DISEASE AND THE ENVIRONMENT

The Laboratory for Infectious Disease and the Environment (LIDE) studies the occurrence, fate and transport, and health effects of human and agricultural zoonotic pathogens in the environment (figs. 1, 2, and 3). The LIDE is an interagency collaborative effort between the U.S. Geological Survey and the U.S. Department of Agriculture-Agricultural Research Service that conducts research to inform decision makers and advance scientific knowledge.

Collaborating with the LIDE

The LIDE collaborates with public agencies and academic researchers in partnerships that span the research process (table 1). Whether leading a project to address a research question or contributing to a larger research effort, the LIDE works cooperatively or independently on all aspects of the research process.

Sample Collection and Analysis

- The LIDE collects and analyzes samples from many **matrices**—groundwater, surface water, runoff, wastewater, sediments, soil, manure, compost, digestate, and air (figs. 4 and 5).
- The LIDE's high-throughput molecular methods (nearly 20,000 assays per year) allow detection, quantification, and subtyping of microorganisms, including 30 pathogens and fecal indicators by **quantitative polymerase chain reaction (qPCR)** (table 2).
- Culture methods**, including standard methods for total coliforms, *Escherichia coli* (*E. coli*), and *Enterococci*, allow detection and quantification of culturable viral and bacterial pathogens and fecal indicators.

Occurrence

What is the distribution and abundance of pathogens in water, soil, manure, and air?

Transport

What actors control pathogen movement, persistence, and degradation?

Health Effects

What are the implications to ecosystem, animal, and human health?

For example...

Are pathogens present in groundwater drinking supplies?

How are pathogens transferred to groundwater in fractured-bedrock settings?

What is the health risk to those who get their drinking water from groundwater wells?



Figure 1. (above left and right) The Laboratory for Infectious Disease and the Environment (LIDE), located in Marshfield, Wisconsin.



Figure 2. (above) The Laboratory for Infectious Disease and the Environment (LIDE) studies the occurrence, fate and transport, and health effects of A, agricultural sources of pathogens (such as manure-based fertilizers) and B, human sources of pathogens (such as wastewater treatment plant discharge).

Figure 3. (left) The Laboratory for Infectious Disease and the Environment (LIDE) approaches every study by formulating research questions from three basic perspectives—what is the occurrence, fate and transport, and health effects of pathogens in the environment?

Table 1. Collaborations with the Laboratory for Infectious Disease and the Environment (LIDE) span the research process and provide access to expertise and support for all aspects of the project.

Developing a research question	The LIDE develops research questions by identifying the relevant concept or problem and then defining the project scope and objectives.
Designing an experiment	The LIDE develops an experimental design that achieves objectives, identifies design aspects that add scientific value, and ensures feasibility through knowledge of methodologies.
Executing a project	The LIDE performs sample collection and analysis and provides training and technical support.
Interpreting data	The LIDE’s subject-matter expertise provides context for results. LIDE also offers support for data interpretation, analysis, and management.
Communicating results	The LIDE produces its own publications and communicates results to stakeholders and decision makers. LIDE also supports collaborator’s communication products by providing necessary information, data, and figures.

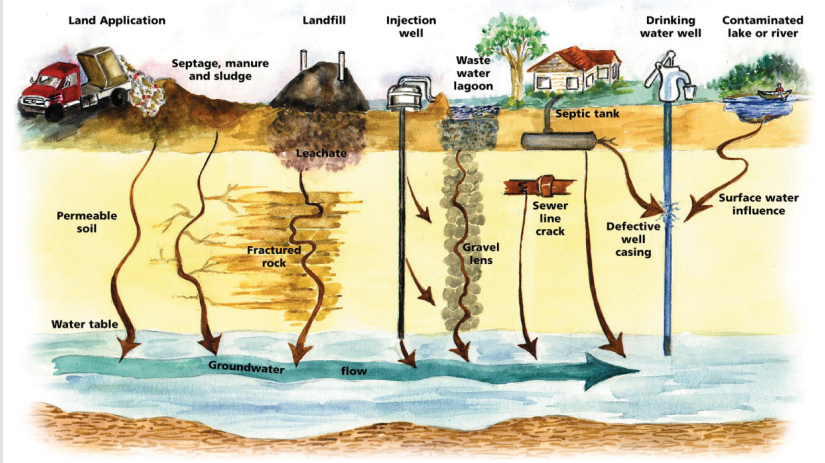
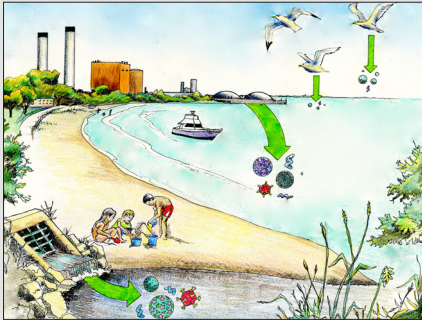


Figure 4. The Laboratory for Infectious Disease and the Environment (LIDE) studies viruses and bacteria in many environmental settings, including groundwater. This figure illustrates many potential sources that can transport pathogens to groundwater. (Modified from Keswick and Gerba, 1980)

Figure 5. The Laboratory for Infectious Disease and the Environment (LIDE) also specializes in recreational-water quality by quantifying pathogen concentrations, identifying environmental factors that influence pathogen occurrence and variability, and estimating health risks. (Illustration from Corsi and others, 2016)



Other LIDE Capabilities

- The LIDE is a biosafety level 2+ approved lab with extensive quality assurance/quality control protocols.
- The LIDE has its own laboratory information management system (LIMS), which provides advanced data management services and quality assurance/quality control documentation.
- The LIDE’s rapid response team performs sample collection and analysis in outbreak situations (page 4, fig. 9).
- The LIDE can provide custom sampling equipment, plans, training, data support, and data interpretation.

Table 2. Assays currently available for quantitative polymerase chain reaction (qPCR) analysis.

Bacteria
<i>Bacteroidales</i> -like Cow M2 and M3.
<i>Bacteroidales</i> -like Hum M2.
Ruminant <i>Bacteroides</i> .
<i>Campylobacter</i> .
<i>Clostridium</i> .
<i>Enterococcus</i> .
Enterohemorrhagic <i>E. coli</i> .
Human <i>Bacteroides</i> .
<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> .
<i>Salmonella</i> .
<i>Staphylococcus</i> .
<i>Streptococcus</i> .
Human viruses
Hepatitis A virus.
Human adenovirus.
Human enterovirus.
Human polyomavirus.
Human rotavirus A.
Norovirus (GI and GII).
Bovine viruses
Bovine adenovirus.
Bovine coronavirus.
Bovine enterovirus.
Bovine polyomavirus.
Bovine rotavirus (group A).
Bovine viral diarrhea virus (type 1 and 2).
Other viruses
Avian influenza.
Hepatitis E virus.
Pepper mild mottle virus.
Rotavirus group C.
Protozoa
<i>Cryptosporidium</i> (<i>parvum</i> and <i>hominis</i>).
<i>Giardia lamblia</i> .
Antibiotic resistance genes
Tetracycline (A, W, X).
Erythromycin.
Sulfanamide.
Class 1 integrons.

Examples of the Laboratory for Infectious Disease and the Environment's (LIDE's) Research

OCCURENCE—Groundwater Viruses in Minnesota Drinking Water Wells

(Stokdyk and others, 2017)

In Minnesota (fig. 1), 74 percent of the population relies on groundwater for drinking water, and 97 percent of community public water systems uses groundwater. Because groundwater wells may be contaminated with viruses that cause waterborne illness or outbreaks (Minnesota Department of Health, 2014), the State legislature requested a targeted study to determine the occurrence of viruses in Minnesota groundwater and the associated risk of illness in the community.

The LIDE determined the occurrence of enteric pathogens in Minnesota public water systems supplied by groundwater. The LIDE completed qPCR analyses on samples that were collected every 2 months for 1 or 2 years from 145 public water wells. The samples were analyzed for human enteric viruses, zoonotic (passed between animals and humans) bacteria, bovine fecal markers, and fecal indicators. LIDE's data analysis will evaluate factors related to pathogen occurrence (such as well characteristics, precipitation, and surrounding land use) and provide a tool for predicting contamination of wells. Data from this study will help determine if virus contamination from groundwater wells presents a human health risk.

LIDE's Role

- **Provided guidance to the State on study design and execution.**
- **Trained collaborators in sample collection techniques, assembled high-volume water sampling units, and provided technical support for field work.**
- **High sample throughput allowed analysis of more than 1,300 water samples for 19 human and bovine viruses, zoonotic bacteria, and fecal indicators.**
- **High-volume sampling techniques allowed detection of low virus concentrations (fig. 6).**
- **The LIDE's LIMS was used to organize data for 1,300 samples, facilitate data analysis, examine data quality, and provide data summaries.**



Figure 6. Well-water sample collection using ultrafiltration.

FATE AND TRANSPORT—Norovirus Outbreak Caused by a New Septic System

(Borchardt and others, 2011)

During the summer of 2007, 211 patrons and 18 employees of a new restaurant in Door County, Wisconsin (fig. 1), were affected by acute gastroenteritis (vomiting and diarrhea). Initial analyses of the restaurant's water were positive for fecal contamination. Investigators used a unique combination of epidemiological, microbial, and hydrogeological evidence that eventually identified a new septic system (constructed according to code and approved by inspection) as the source of the contamination.

First, epidemiological studies identified that patients who had consumed water-based items like drinking water, ice, and washed salad were more likely to become ill. Second, the LIDE completed the microbial investigation, evaluating water samples from restaurant taps, raw well water, and septic effluent by using qPCR analysis to identify which pathogens were present and which water sources had been contaminated. Norovirus genotyping also was completed by the LIDE and results determined that the norovirus had traveled through the groundwater from the septic system to the well and to the restaurant's water taps. Finally, hydrogeological studies used two fluorescent dyes to track flow paths and travel times between the restaurant, septic system, and several local domestic wells (fig. 7). Both dyes were detected in the restaurant's water supply well, indicating that effluent from the septic tanks and infiltration field was moving through the groundwater and reaching the restaurant's well.

LIDE's Role

- **The LIDE's rapid response to the outbreak provided timely sample collection and analysis.**
- **The LIDE worked collaboratively with State, County, and university departments and agencies to identify the source of the outbreak.**

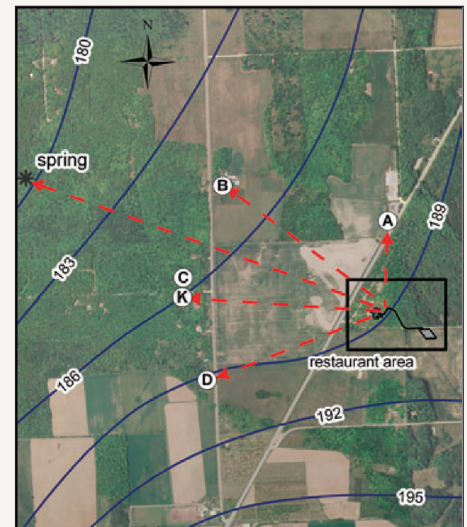


Figure 7. Map showing point-to-point dye detections in domestic wells near the restaurant where the outbreak occurred, Door County, Wisconsin (fig.1). Potentiometric-level contours, in meters above sea level, represent a hypothetical water-table surface showing which direction water would potentially flow. (From Borchardt and others, 2011)



HEATH EFFECTS—Evaluating the Risks of Airborne Pathogens from Manure Irrigation

(Burch and others, 2017)

Manure irrigation, which is the application of liquid animal manure by traveling gun or center pivot irrigation (fig. 8), is increasing in Wisconsin and other states. However, the risk of airborne pathogen transmission from manure to humans during spray irrigation is not understood.

To determine how pathogens can spread during manure irrigation, the LIDE measured air concentrations of manure-borne microorganisms during 21 full-scale manure irrigation events on 3 Wisconsin dairy farms. Samples were collected at multiple downwind distances—typically as much as 700 feet—and were analyzed using qPCR and bacterial cultures.

To complete the quantitative microbial risk assessment, the measured concentrations of manure-borne microorganisms in the air were then used with pathogen dose-response curves and with information related to the length and intensity of human exposure to manure irrigation. The quantitative microbial risk assessment resulted in predictions of the risk of human illness because of exposure to pathogens in the irrigation spray, which can be used by decision makers to inform selection of risk mitigation policies.

LIDE's Role

- Developed study design and sampling strategy for meeting policy-makers' information needs.
- Fostered positive working relationships with the producers who provided field-sampling opportunities.
- Broadened LIDE field-sampling techniques to include airborne microorganisms.
- Developed additional bovine-specific qPCR assays.



Figure 8. (above) Photographs showing *A*, traveling-gun manure irrigation system at a Wisconsin farm and *B*, Laboratory for Infectious Disease and the Environment (LIDE) scientists collecting an air sample that will be cultured for bacterial pathogens.

Figure 9. (background below) A U.S. Geological Survey scientist sampling well water at a poultry farm for the avian influenza virus.

References Cited

- Borchardt, M.A.; Bradbury, K.R.; Alexander, E.C., Jr.; Kolberg, R.J.; Alexander, S.C.; Archer, J.R.; Braatz, L.A.; Forest, B.M.; Green, J.A.; and Spencer, S.K., 2011, Norovirus outbreak caused by a new septic system in a dolomite aquifer: *Ground Water*, v. 49, no. 1, p. 85–97.
- Burch, T.R.; Spencer, S.K.; Stokdyk, J.P.; Kieke, B.A., Jr.; Larson, R.A.; Firnstahl, A.D.; Rule, A.M.; and Borchardt, M.A., 2017, Quantitative microbial risk assessment for spray irrigation of dairy manure based on an empirical fate and transport model: *Environmental Health Perspectives*, v. 125, no. 8, accessed September 18, 2017, at <https://ehp.niehs.nih.gov/chp283/>.
- Corsi, S.R., Borchardt, M.A., Carvin, R.B., Burch, T.R., Spencer, S.K., Lutz, M.A., McDermott, C.M., Busse, K.M., Kleinheinz, G.T., Feng, X., and Zhu, J., 2016, Human and bovine viruses and bacteria at three great lakes beaches—Environmental variable associations and health risk: *Environmental Science & Technology*, v. 50, no. 2, p. 987–995.
- Keswick, B.H., and Gerba, C.P., 1980, Viruses in groundwater: *Environmental Science & Technology*, v. 14, no. 11, p. 1290–1297.
- Minnesota Department of Health, 2014, Clean water fund—Keeping Minnesota healthy: Minnesota Department of Health Five Year Progress Report 2010–2014, 11 p., accessed August 6, 2014, at <https://www.leg.state.mn.us/docs/2015/other/150450.pdf>.
- Stokdyk, J.P., Anderson, A.C., Rezania, L.W., Spencer, S.K., Firnstahl, A.D., Robinson, T.J., Borchardt, M.A., 2017, Occurrence of human and bovine pathogens and fecal markers in non-disinfected drinking water from community and non-community wells in Minnesota [abs.]: University of North Carolina Water Microbiology Conference, May 15–17, 2017, accessed November 8, 2018, at <https://waterinstitute.unc.edu/files/2016/10/WaterMicro-HRWM-Abstract-Book.pdf>.

For more information about LIDE visit
<https://www.usgs.gov/LIDE> or contact:

Mark Borchardt, LIDE Director (USDA)
Mark.Borchardt@ars.usda.gov
715-387-4943

Joel Stokdyk, LIDE USGS Liaison
jstokdyk@usgs.gov
715-384-9673



ISSN 2327-6916 (print)
ISSN 2327-6932 (online)
<https://doi.org/10.3133/fs20183079>