Airborne Pathogen Transport from Spray Irrigated Manure Liquids - Background Information

Mark Borchardt, PhD, and Susan Spencer

USDA – Agricultural Research Service

Jan Altmann, Jordan Gonnering and Hana Millen

US Geological Survey Wisconsin Water Science Center

Marshfield, Wisconsin

Manure's Double-Edged Sword

Manure as Asset

Manure field-application is a cost-effective and sustainable approach for optimal soil tilth and fertility

Manure as Liability

Manure may contain pathogens harmful to both humans and livestock

Societal goal: Maximize the beneficial uses of manure while minimizing environmental pathogen transmission

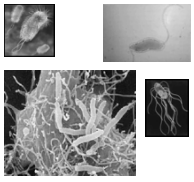
Manure Irrigation Environmental Tradeoffs

Pathogen drift and transmission

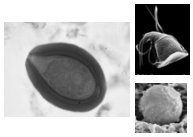
- Reduced road traffic
- Minimize runoff
- Maximize nutrient utilization
- Minimize groundwater contamination

Pathogens in Cattle Manure

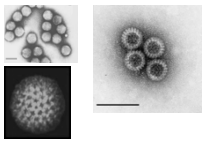
Bacteria (e.g., *Campylobacter*, *Salmonella*, *E. coli* O157:H7)



Protozoa, (e.g., *Cryptosporidium*, *Giardia*, *Eimeria)*



Viruses (e.g., adenovirus, enterovirus, rotavirus)



Estimates of Enteric Illness Attributable to Contact with Animals and Their Environments in the United States

- 445,213 /3.2 million illnesses (14%) from animal contact

Organism	% from animal contact	Annual # illnesses	Annual # hospitalizations	Annual # deaths
Campylobacter species	17%	187,481	1,877	17
ST E. coli	14	16,057	230	2
Non-typhoid Salmonella	11	127,155	2,392	47
Cryptosporidium species	16	113,344	412	7

Centers for Disease Control and Prevention: Hale et al. 2012, CID, 54:S472-79.

Cryptosporidium parvum

- Scours in calves
- Responsible (with C. hominis) for largest waterborne disease outbreak in US history
- Severe diarrhea 21 days median duration
- 7-22% of patients hospitalized
- Deadly infection in AIDS patients and immunocompromised
- Infected children have reduced growth

Toxin producing *E. coli*

- E. coli O157:H7, the Jack-in-the-Box bug
- No disease in cattle
- Severe diarrhea; 4% of cases develop kidney failure
- 73,000 cases, 60 deaths/year in US
- Walkerton, Ontario outbreak

Salmonella enterica

- In cattle, diarrhea, milk drop, abortion, rapid death in calves
- In people, diarrhea, cramps, fever
- Can move from the intestine to bloodstream, bone, and urinary tract
- 1.4 million cases, 600 deaths/year in US
- Growing antibiotic resistance
- Drinking raw milk presents high risk of infection

Campylobacter jejuni and C. coli

- Most common cause of bacterial gastrointestinal illness in the US
- Debatable among veterinarians whether a cattle pathogen
- Severe diarrhea, potential complications with liver, heart, other organs
- Causes Guillain-Barré syndrome, acute paralysis

Other zoonotic pathogens in cattle manure - Infrequent human health effects

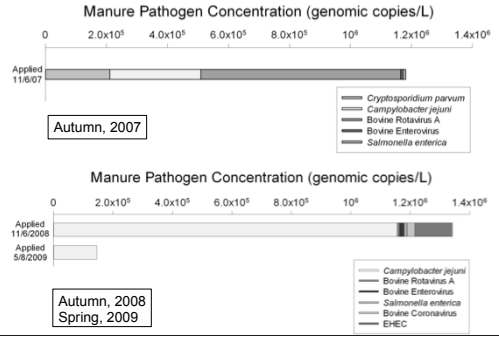
Microsporidia	Leptospira species
Brucella species	Listeria monocytogenes
Bacillus anthracis	Mycobacterium bovis
Clostridium perfringens	Apthovirus (foot and mouth disease)
Coxiella burneti	

Sources
Dungan, RS. 2010. J. Anim. Sci. 88:3693-3706
Atwill, ER. Et al. 2012 . NRCS Technical Note No. 9

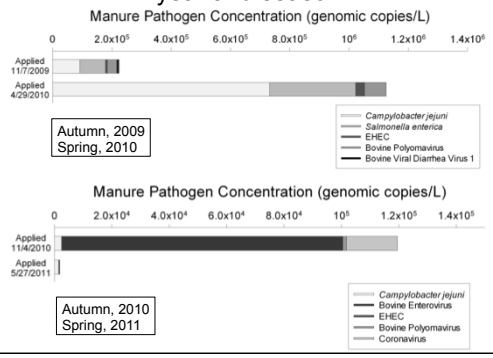
Pathogen traits favoring transmission

- Released to the environment in high numbers in feces
- Long survival times in the environment
- Low dose required for infection
- Capable of multiplying in the environment (i.e., no host required)

Pathogens in manure from a single farm by year and season



Pathogens in manure from a single farm by year and season



Autumn, 2009
Spring, 2010

Autumn, 2010
Spring, 2011

Pathogen traits favoring transmission

- Released to the environment in high numbers in feces
- Long survival times in the environment
- Capable of multiplying in the environment (i.e., no host required)
- Low dose required for infection

Infectious Dose

Definition: The quantity of pathogen necessary to caise an infection in a susceptible person

Pathogen	Infectious Dose (estimated)
Salmonella	100 – 1,000,000 cells
Campylobacter[1]	500 cells
E. Coli O157:H7	10 – 100 cells
Cryptosporidium[1]	10 oocysts

[1] Kothary and Babu. 2001 J Food Safety 21:49-73.

Environmental factors favoring pathogen inactivation

- Warm temperatures, greater than 68°F
- Dry desiccating conditions
- Ultraviolet radiation from sunlight
- Freeze-thaw cycles
- Low organic content
- Native microbial communities

INACTIVATION OF DAIRY MANURE-BORNE PATHOGENS BY ANAEROBIC DIGESTION AND BEDDING RECOVERY UNITS

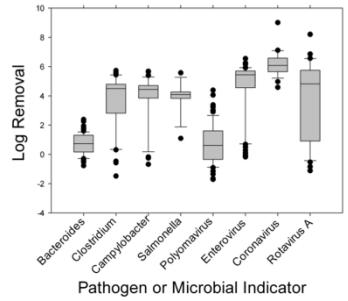
Mark Borchardt, Susan Spencer, and Spencer Borchardt
USDA –Agricultural Research Service
USGS Wisconsin Water Science Center

Becky Larson and Asli Ozkaynak
UW-Madison Biological Systems Engineering

Study Overview

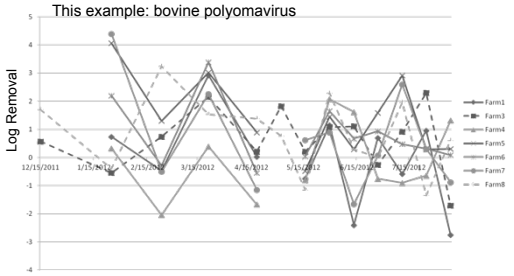
- Samples collected from nine farms: two with complete mix digesters, five with plug flow digesters, and two that have only screw press bedding recovery units.
- Samples collected approximately every two weeks for eight months, December 2011 – August 2012.
- Four sampling points: 1) Pre-digest (i.e., manure); 2) Post-digest; 3) Solids after separation; 4) Liquid after separation
- All pathogens and indicators measured by qPCR (i.e., measuring genomes) and reported as genomic copies per gram.

Digester Effectiveness in Removing Pathogens Differs by Pathogen Type



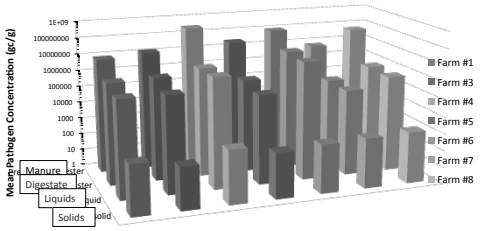
Digester Effectiveness in Removing Pathogens is Highly Variable Over Time

This example: bovine polyomavirus



After Digestion and Separation the Majority of Pathogens are in the Liquid Fraction

This example: bovine polyomavirus



Study Limitations

- Sampling frequency was not based on digester retention time therefore the manure and digestate samples are not truly coupled, particularly for the plug-flow digesters.
- Measured inactivation of pathogen/indicator genomes; this is not a measure of infectivity or viability.
- Pathogen concentrations in many samples were near the assay limit of detection, which reduces accuracy of the log removal estimate

Preliminary Conclusions

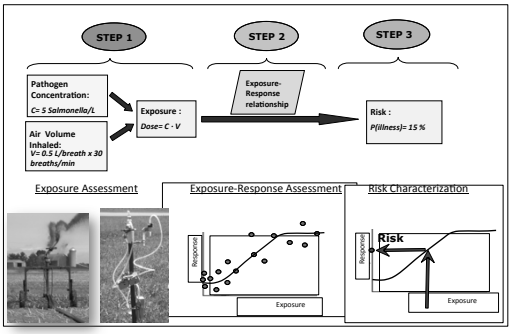
- Full-scale anaerobic digesters reduced pathogen levels by 90% to 99.9%
- Removal efficiency varied by pathogen type, farm, and time
- After digestion and separation of the digestate, the liquid fraction contained the majority of pathogens.
- Separation of undigested manure by screw press bedding recovery units resulted in the liquid fraction containing the majority of pathogens.
- Although the solids fraction contained fewer pathogens, the concentration could still be above the infectious dose, particularly for calves
- Heating the solids should result in complete pathogen inactivation but this was not always the case

Does Anaerobic Digested Manure have Reduced Health Risks?

<u>Findings and perspectives to keep in mind…</u>

- Pathogen types and concentrations in manure (i.e., the herd) are highly variable over time
- Pathogen inactivation by anaerobic digestion is highly variable
- Because pathogen concentrations in manure can be very high, a 99% reduction (i.e., 2-log removal) does not mean pathogen levels become low
- 99% of the pathogens in the digestate after separation partition into the liquid fraction
- Digesters are designed to produce methane, not inactivate pathogens.

Overview of Quantitative Microbial Risk Assessment (QMRA)



STEP 1 Exposure Assessment

The population's exposure to pathogens present in air (i.e., dose) depends on:

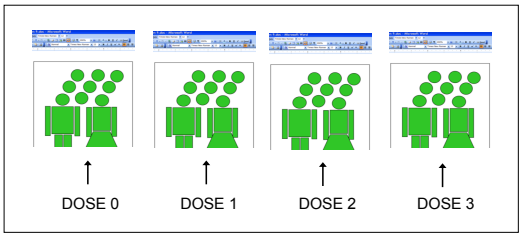
- Pathogen concentration in air
- Breathing rate
- Lung tidal volume
- Length of time exposed
- Age and health status of exposed population
- For gastrointestinal pathogens, the fraction of inhaled material that is ingested

Example
0.4 *Campylobacter*/L air x 30 breaths/min x 0.5 L/breath x 60 min/hr x 2 hour exposed x 25% ingested = 180 *Campylobacter* ingested

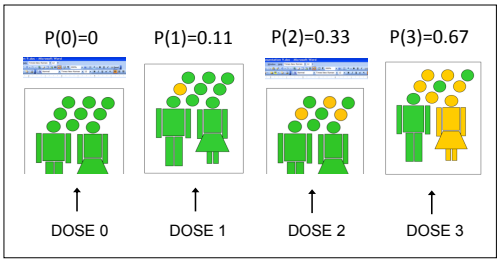
STEP 2 Exposure-Response Assessment

- Links the dose of etiological agent to health effect
- EXPOSURE: how many pathogens are ingested *(PFUs, MPN, genomic copies)*
- RESPONSE: how many people get sick due to ingesting a specific dose *(probability of illness)*

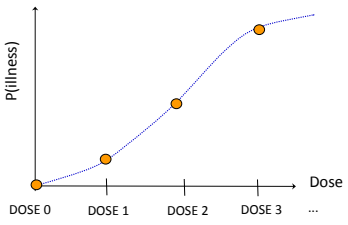
STEP 2 Exposure-Response Assessment: Traditional Methods use Human Feeding Studies



STEP 2 Exposure-Response Assessment: Response Curve from Feeding Studies



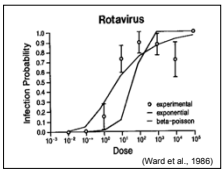
STEP 2 Exposure-Response Assessment: Development of Response Curve using Feeding Studies



STEP 2

Exposure-Response Relationship

Some common Dose-Response equations:



Beta-Poisson
(e.g. rotavirus):

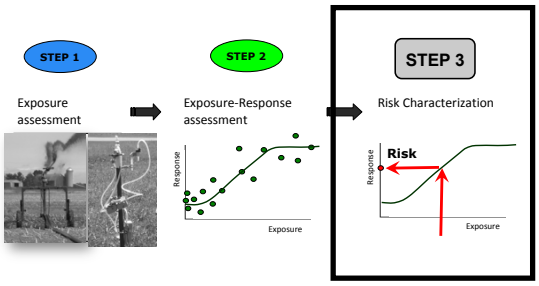
$$P_i(D) = 1 - \left(\frac{D}{N_{50}} \cdot (2^{\frac{1}{\alpha}} - 1)\right)^{-\alpha}$$

Exponential
(e.g. poliovirus, echovirus, adenovirus):

$$P_i(D) = 1 - e^{-rD}$$

STEP 3

Risk Characterization

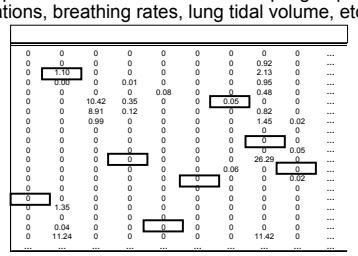


STEP 3

Risk Characterization: Monte Carlo simulation

Generate exposure distribution: random sampling of pathogen concentrations, breathing rates, lung tidal volume, etc

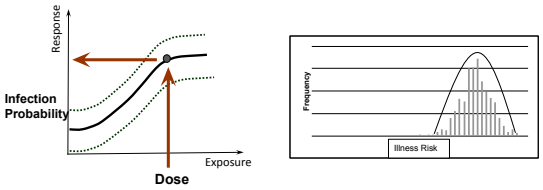
Data matrix



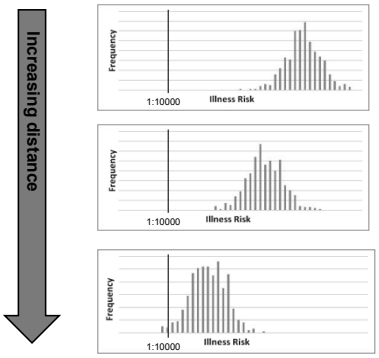
STEP 3

Risk Characterization

Input pathogen dose into Exposure-Response equation → Repeat 100,000 times to generate frequency distribution of illness risk



Calculate risk at increasing distances from manure irrigation until illness risk is below an acceptable threshold



Considerations for Airborne Pathogen QMRA

Other transmission routes besides inhalation
- Fomites
- Water
- Food

Vulnerable populations
- Elderly
- Children
- Immunocompromised or immunosupressed (4% of US population)

Other species
- Nearby dairy herds
- Wildlife

Why Didn't Becky Become Ill?

- No pathogens in manure able to infect humans
- Pathogen concentration in manure was too low
- Pathogen concentration in air was too low
- Exposure time in manure spray was too short
- Excellent immune status
- Probability, illness did not happen this time

Top Three Justifications for Ignoring Hygiene & Sanitation

"I've been working with manure for years and never been sick."
- Situations change: Different pathogens, different cows in herd, different immunity, different people in your life

"What doesn't kill you makes you stronger."
- Assumes you won't get killed
- Assumes exposure can be controlled to just the right amount for increased immunity
- Variation of the theme: Survival of the fittest; the weak should be removed

"Our world is too clean."
- The Hygiene Hypothesis appears true
- By number of cells we are more microbe than human
- But the justification ignores the distinction between pathogens and "friendly" commensal bacteria.

Caution but Not Over-Reaction

- The old paradigm of pathogen exposure = disease no longer holds
- Host-pathogen interaction is an active research area

Story 1: Edward Jenner and the dairy maids in the 18th century

Story 2: Newlyweds and their Campylobacter honeymoon

Story 3: "Negative" control toxigenic E. coli isolated from lab tech

Outbreaks Happen…

Listeria monocytogenes outbreak from eating contaminated cantaloupe

August – December 2011, 28 states

146 sick, 30 dead, one miscarriage

Lawsuits are expected to result in settlements of $125 million to $150 million

Manure on a truck used to haul culled melons to a cattle operation suspected to have introduced *Listeria* into the melon packing shed.

Questions? Comments?