INACTIVATION OF DAIRY MANURE-BORNE PATHOGENS BY ANAEROBIC DIGESTION

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Travelling Gun Irrigation with Liquid Dairy Manure, September 26, 2012

Manure's Double-Edged Sword

Manure as Asset

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

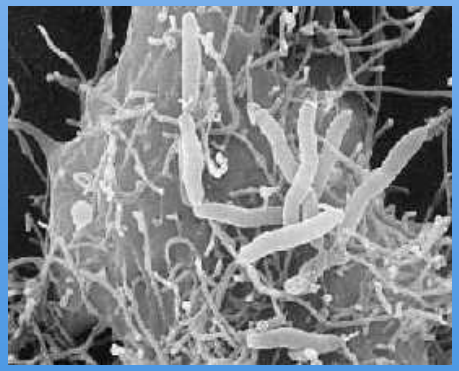
Manure as Liability

Manure may contain pathogens harmful to both humans and livestock

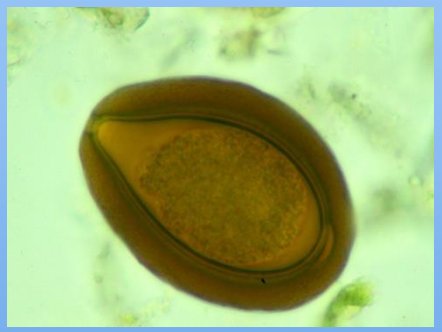
Societal goal: Maximize the beneficial uses of manure while minimizing environmental pathogen transmission

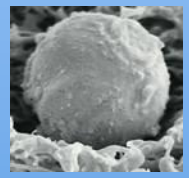
Pathogens in Cattle Manure

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

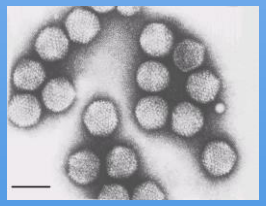


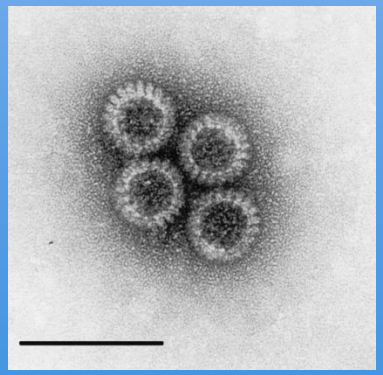
Protozoa, (e.g., *Cryptosporidium*, *Giardia*, *Eimeria*)





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





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

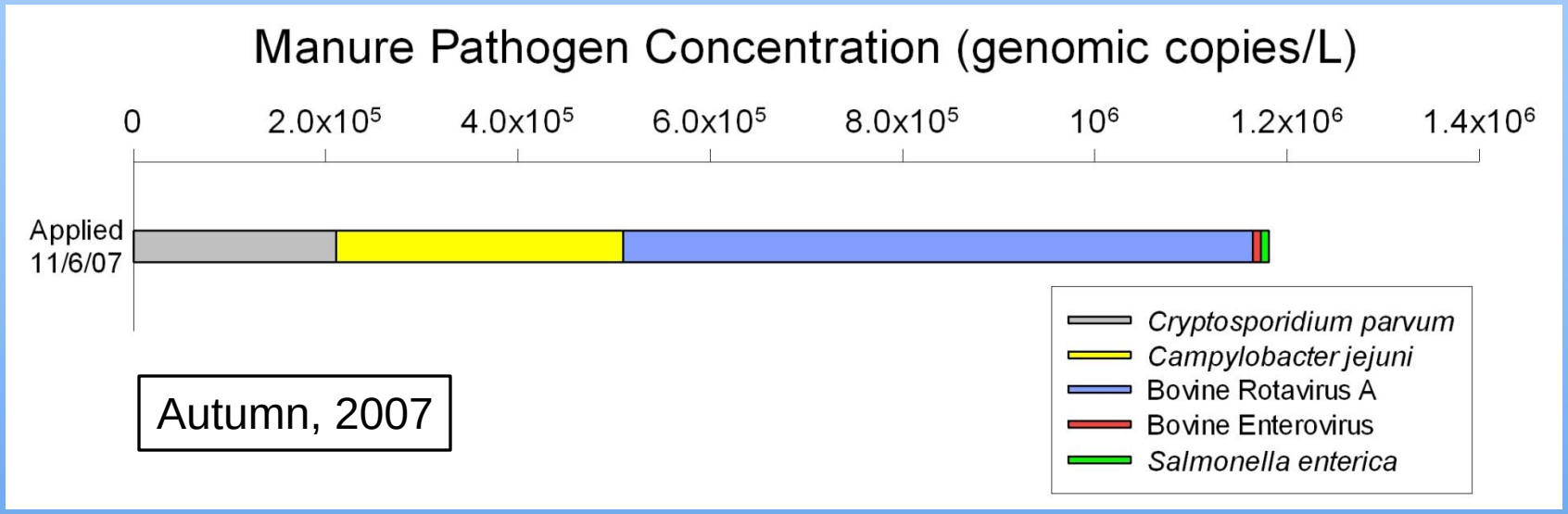
- Most common cause of bacterial gastrointestinal illness in the US
- Debatable among veterinarians whether a cattle pathogen
- Severe diarrhea, potential complications with liver, heart, other organs
- Causes Guillain-Barré syndrome, acute paralysis

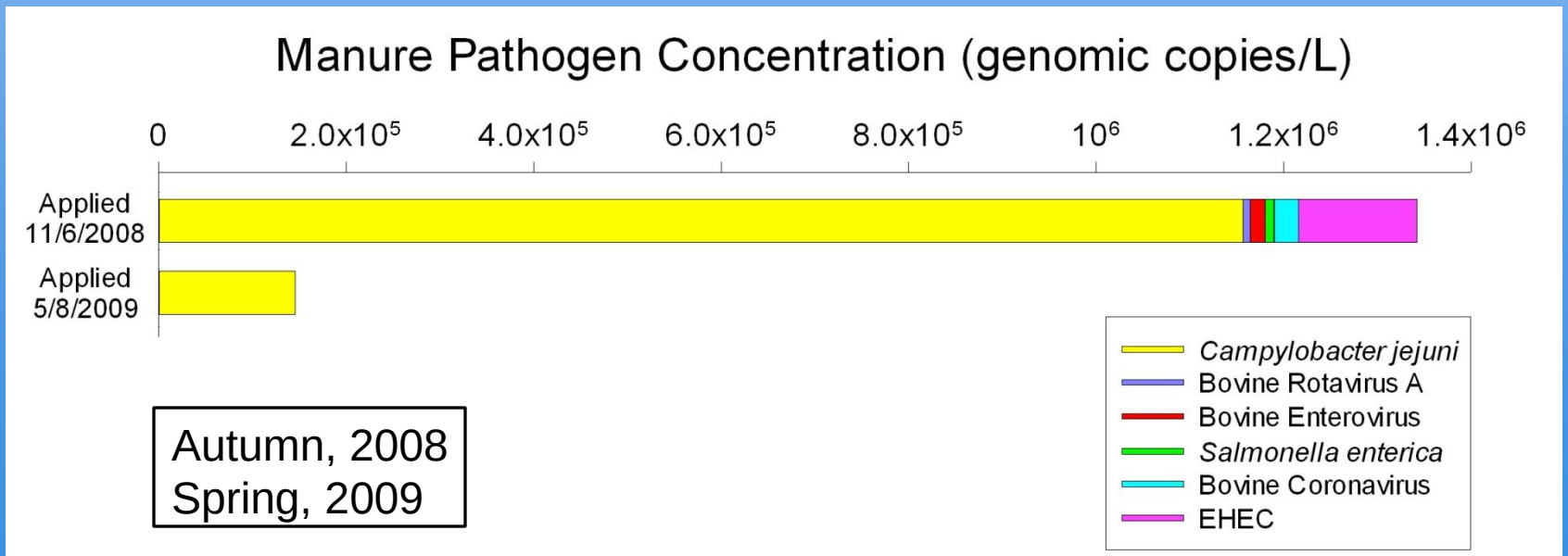
Other zoonotic pathogens in cattle manure - Infrequent human health effects

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

Pathogens in manure from a single farm by year and season





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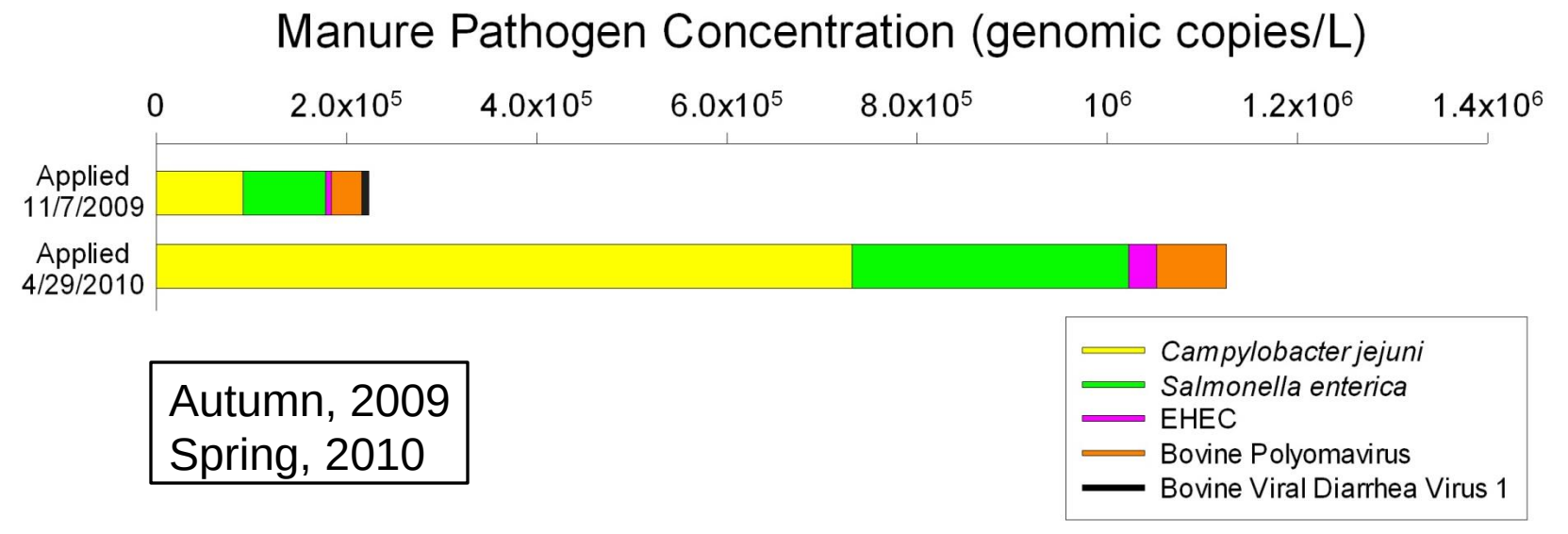
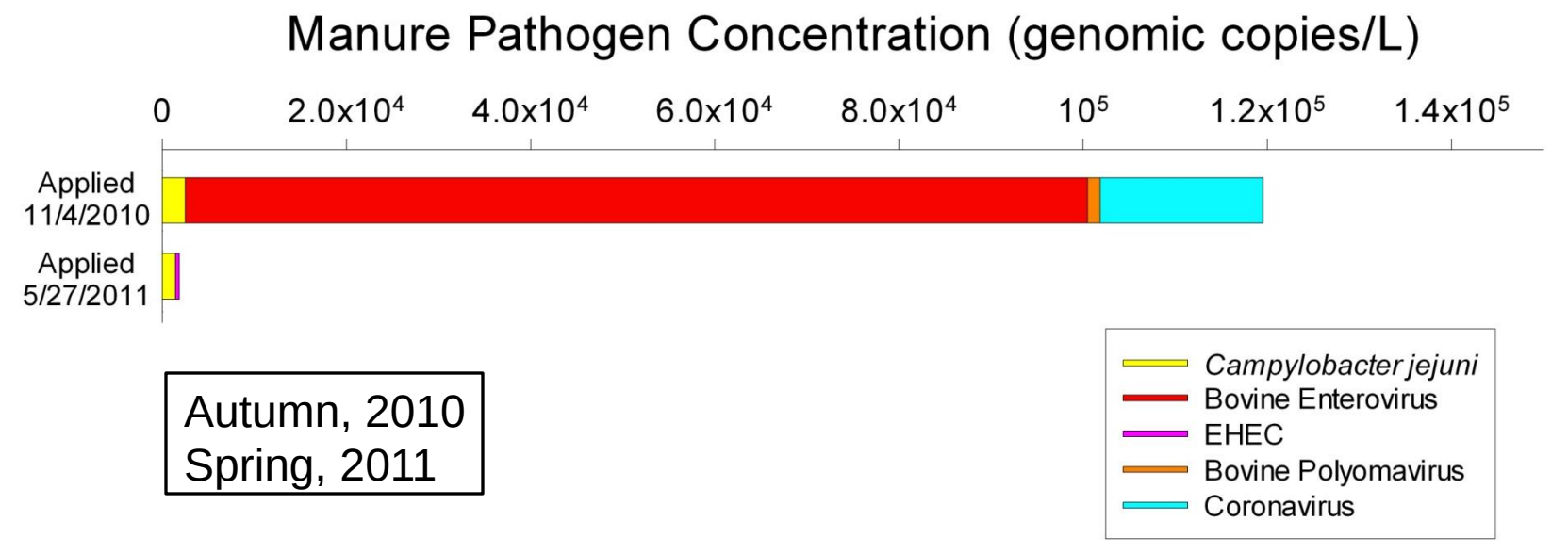




Anaerobic Digesters …

- Produce methane for generating electricity and heat
- And…
- Might, as an added side-benefit, inactivate/destroy pathogens in manure that can potentially pose a risk to the health of humans and livestock

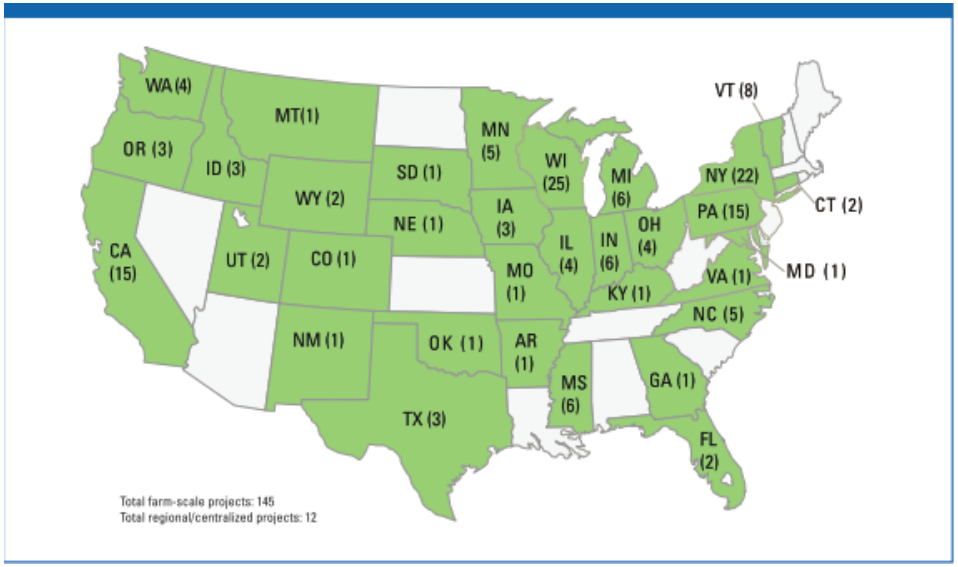
Operational principle of sanitation since Louis Pasteur

•Reducing the number of pathogens reduces the level of exposure which reduces the risk of infection

•Principle works at small scale (hand sanitizers) to large scale wastewater and drinking water treatment plants

Use in the USA

- EPA/USDA AgSTAR program estimates 157 operational digesters as of July 2010
  - This is a 5-fold increase from 2002
- Most are located on dairy farms
- Midwest, West, and Northeast



Current Knowledge

- Mesophilic anaerobic digestion can reduce by 99% to 99.9% the levels of several microbes: E. coli, fecal coliform bacteria, fecal streptococci, Listeria monocytogenes, Salmonella senttenberg.
- Thermophilic anaerobic digestion is more effective than mesophillic digestion in removing pathogens
- Pathogen removal depends on temperature, pH, and retention period

Key Knowledge Gaps

- Pathogen inactivation in full-scale operational digesters.
- Pathogen-specific inactivation rates for the complete array of pathogens potentially present in dairy manure.
- Operational parameters of full-scale digesters most important for pathogen removal
- Pathogen levels in final solids component used for cow bedding

Study Objectives

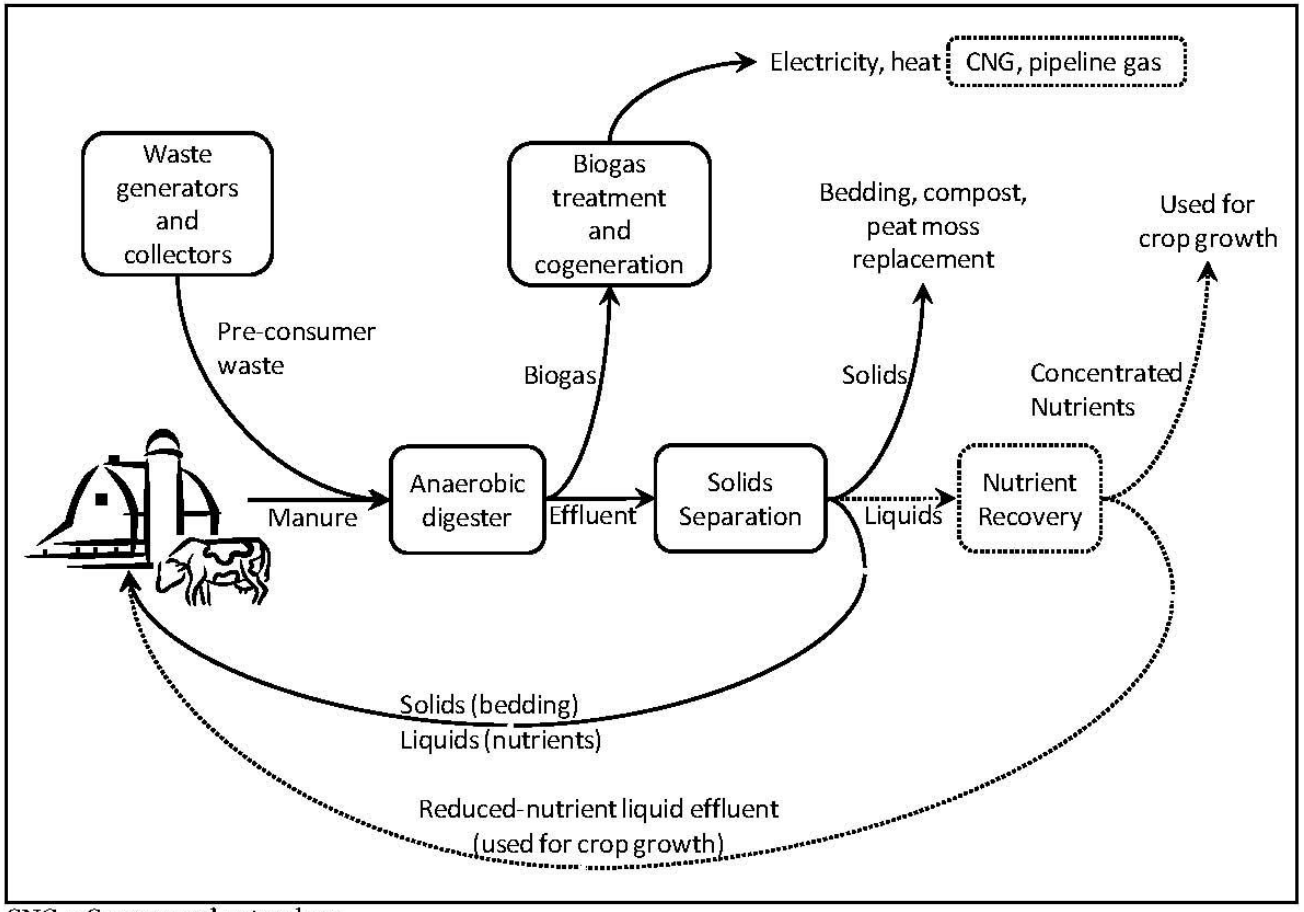
- Measure pathogen inactivation by farm-scale anaerobic digestion of dairy manure
- Evaluate several factors for their effect on pathogen inactivation (pathogen type, farm, time of year)
- Determine proportion of surviving pathogens in digestate liquid and solid fractions after separation
- Determine effect of bedding recovery units (no digestion) on fractionation of pathogens into liquids and solids

Study Overview

- Nine farms in study, seven farms with anaerobic digesters and two that have only screw press bedding recovery units.
- Anaerobic digesters all mesophilic, two complete mix digesters and five plug flow
- Samples collected approximately every two weeks for eight months, January – 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.

Study Sampling Locations

Figure 3. Dairy AD system with added feedstocks and potential nutrient recovery



CNG = Compressed natural gas

Source: Washington Dairies and Digesters, Washington State Dept of Agriculture, AGR PUB 602-343, October 2011

Pathogens and Indicators Analyzed

Protozoa	Bovine Viruses
Cryptosporidium parvum	Rotavirus groups A and C
Giardia lamblia	Enterovirus
	Adenovirus
	Polyomavirus
Bacteria	Bovine viral diarrhea virus types 1 and 2
Enterohemorrhagic *E. coli*	Coronavirus
Campylobacter jejuni	
Salmonella enterica	Indicators
Clostridium perfringens	Bovine bacteroides
Mycobacterium avium paratuberculosis	Bacteroides-like M3
	Bacteroides-like M2

Pathogens Detected during Study

Protozoa	Bovine Viruses
Cryptosporidium parvum	Rotavirus group A
Giardia lamblia	Rotavirus group C
	Enterovirus
	Adenovirus
	Polyomavirus
	Bovine viral diarrhea virus type 1
Bacteria	Bovine viral diarrhea virus type 2
Enterohemorrhagic *E. coli*	Coronavirus
Campylobacter jejuni	
Salmonella enterica	Indicators
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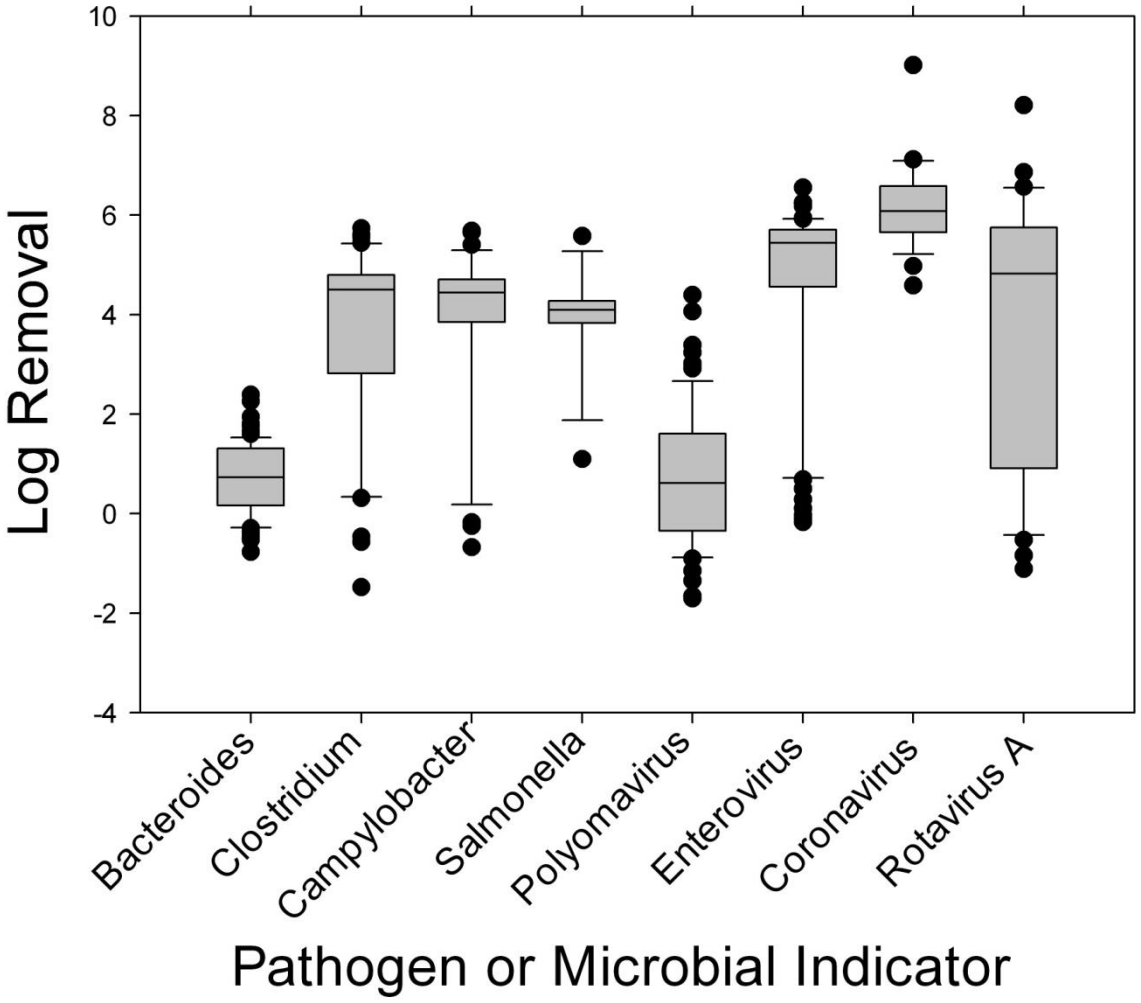
Logarithm (Log) Removal

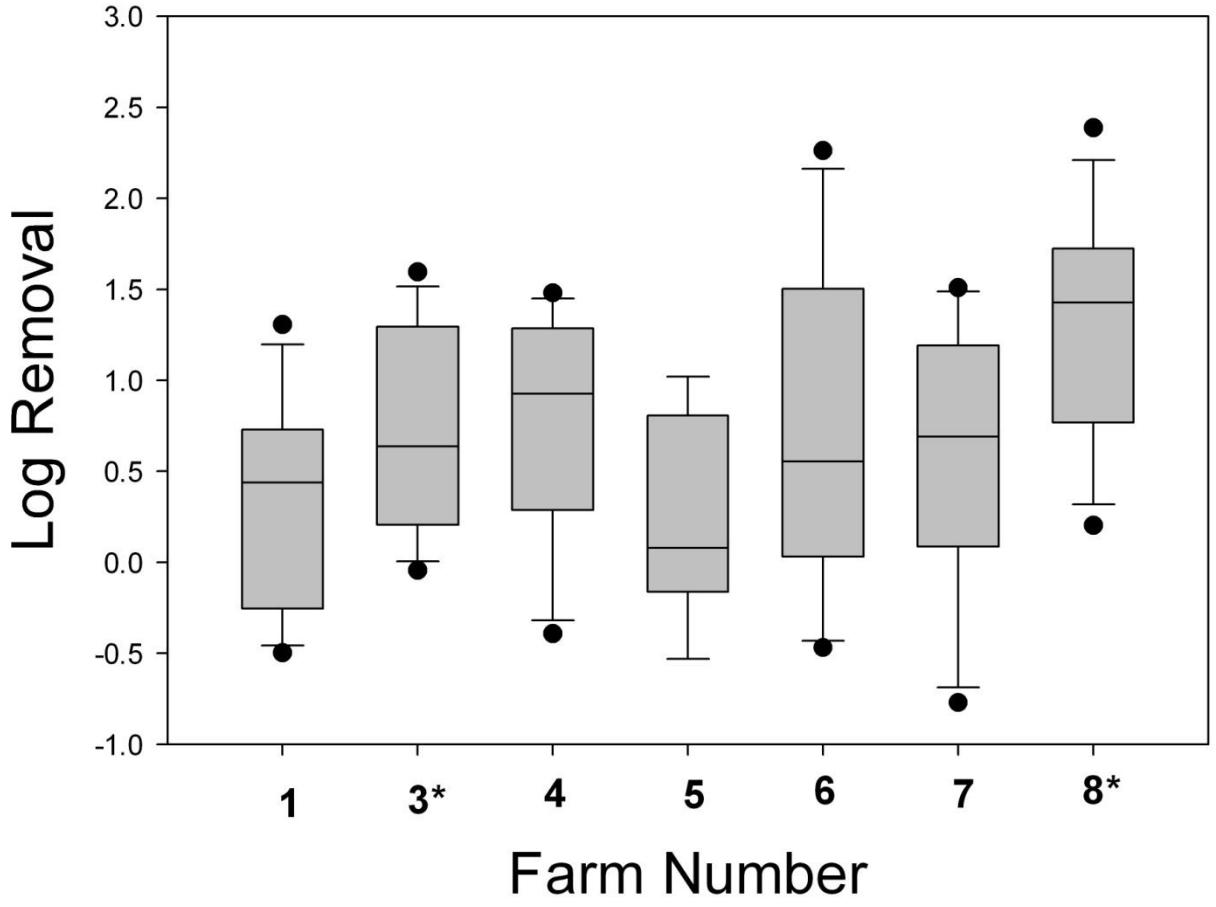
- The logarithm of the pathogen concentration in the manure minus the logarithm of the pathogen concentration in the digestate
- 1 log removal = 90% reduction
- 2 log removal = 99% reduction
- 3 log removal = 99.9% reduction, etc.

For plug flow digesters the digestate concentration used in the log removal calculation was offset by two sampling periods from the manure concentration to account for retention time of approximately 21 days.

Digester Removal by Pathogen Type

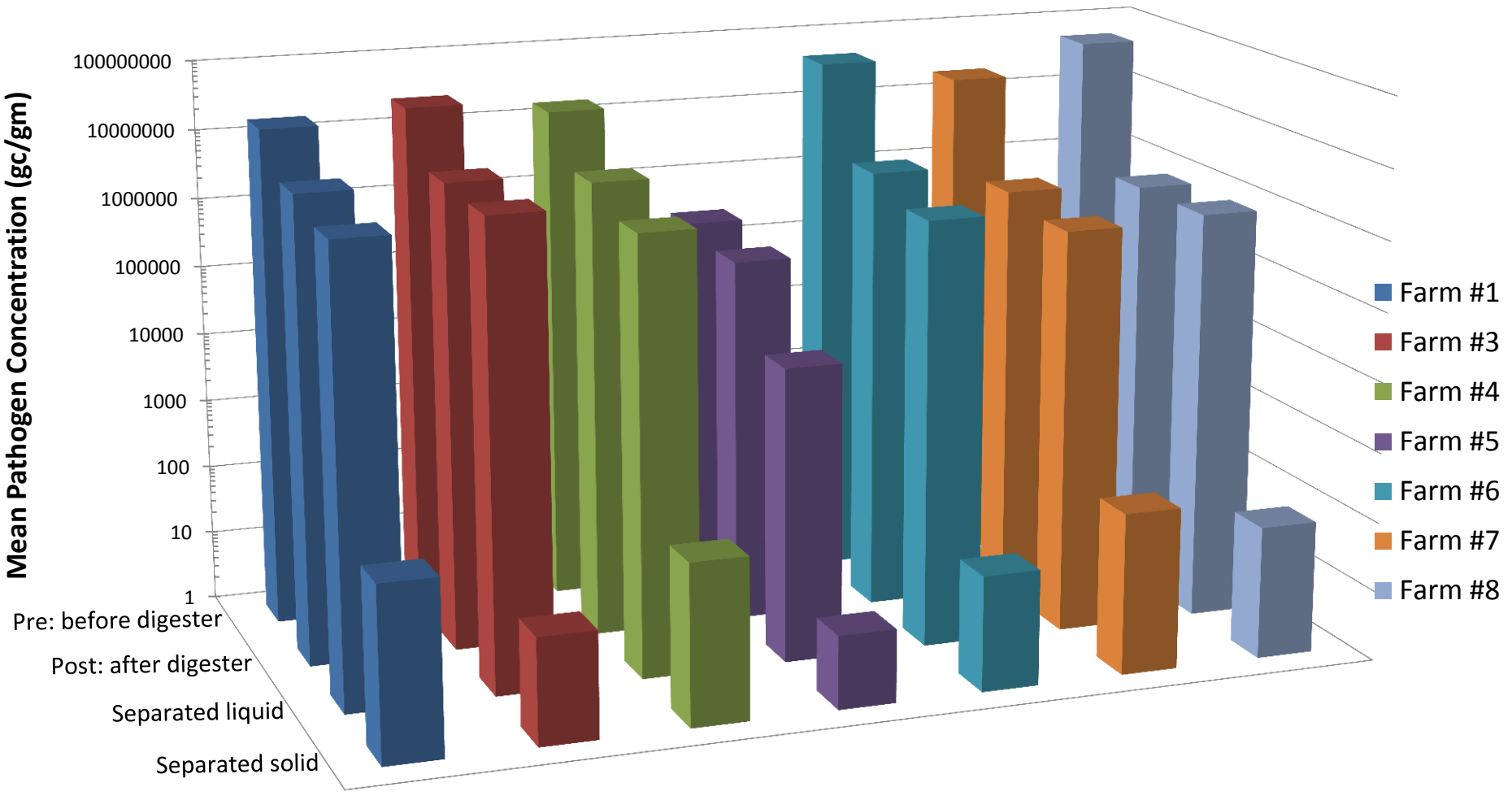


Digester Removal of Bacteroides by Farm

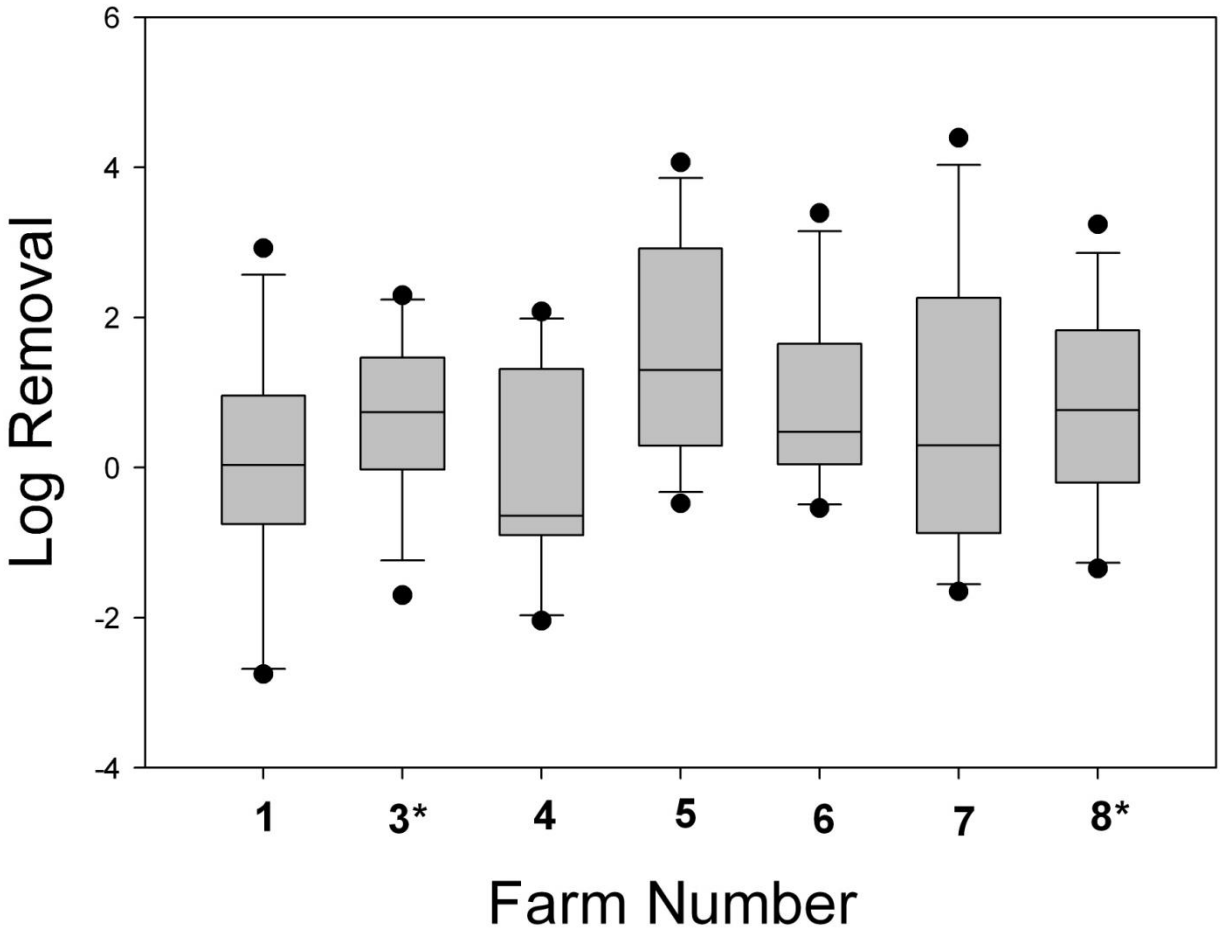


* Continuous mix digester

Bovine Bacteroides

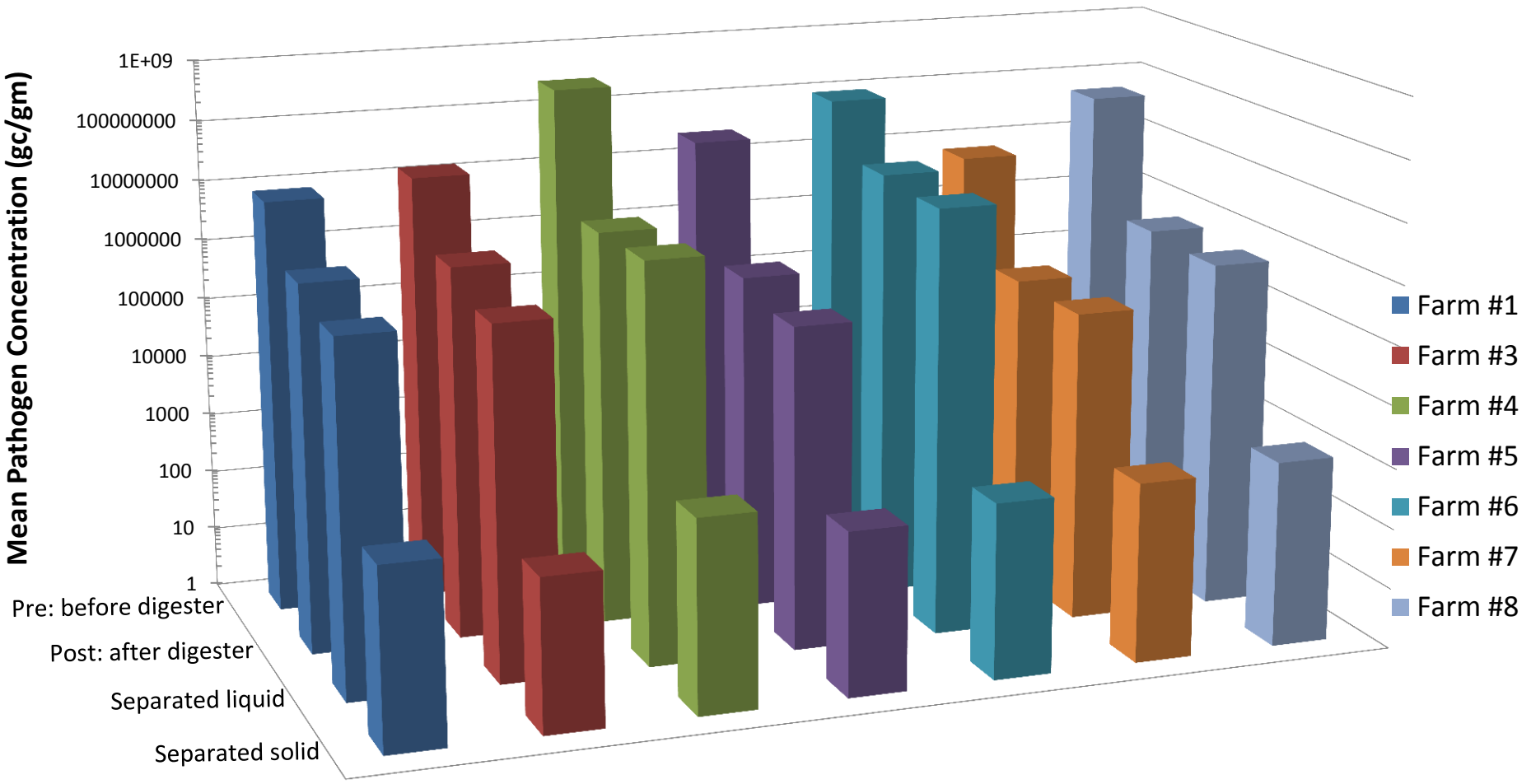


Digester Removal of Polyomavirus by Farm

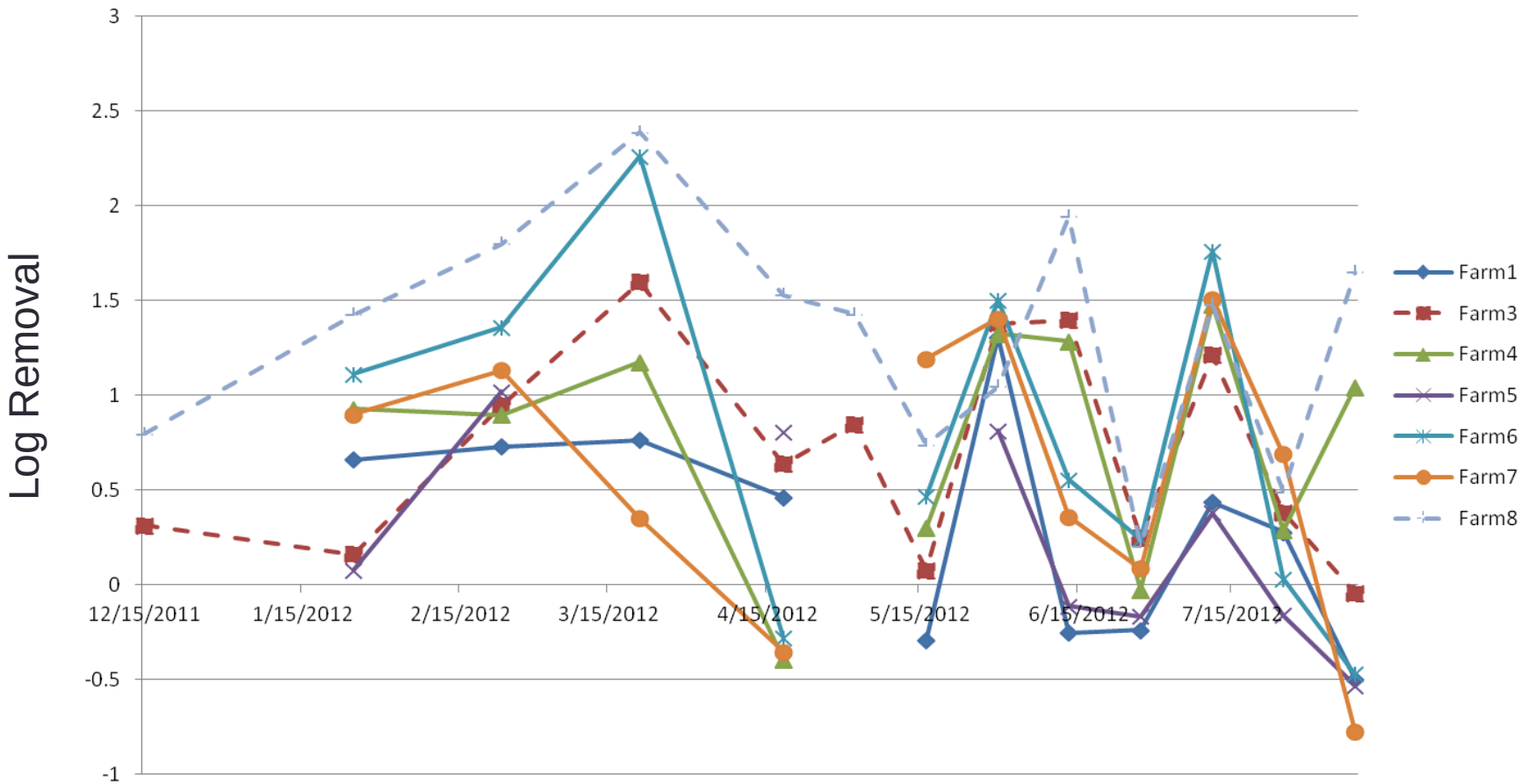


* Continuous mix digester

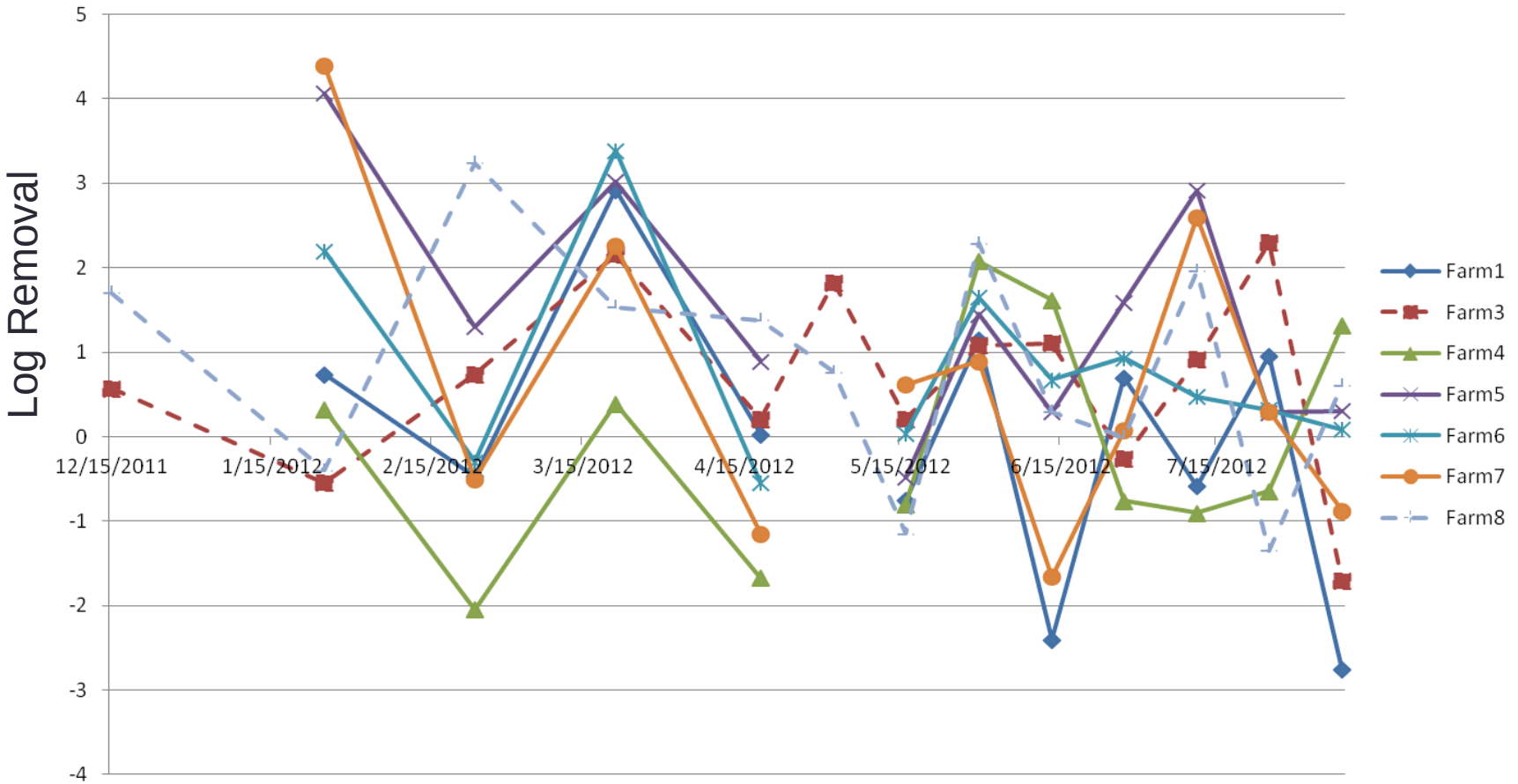
Bovine Polyomavirus



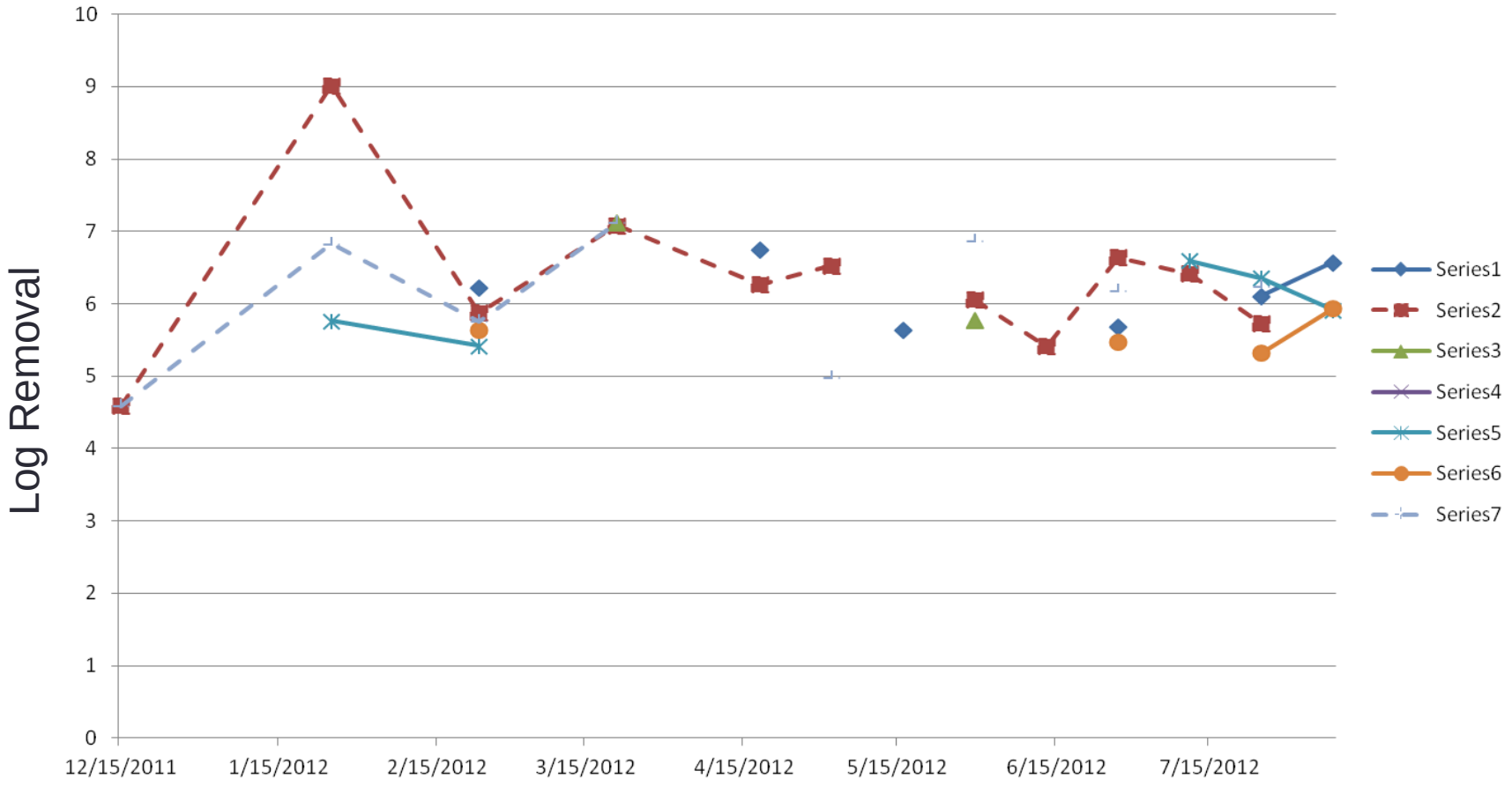
Digester Removal of Bacteroides over Time



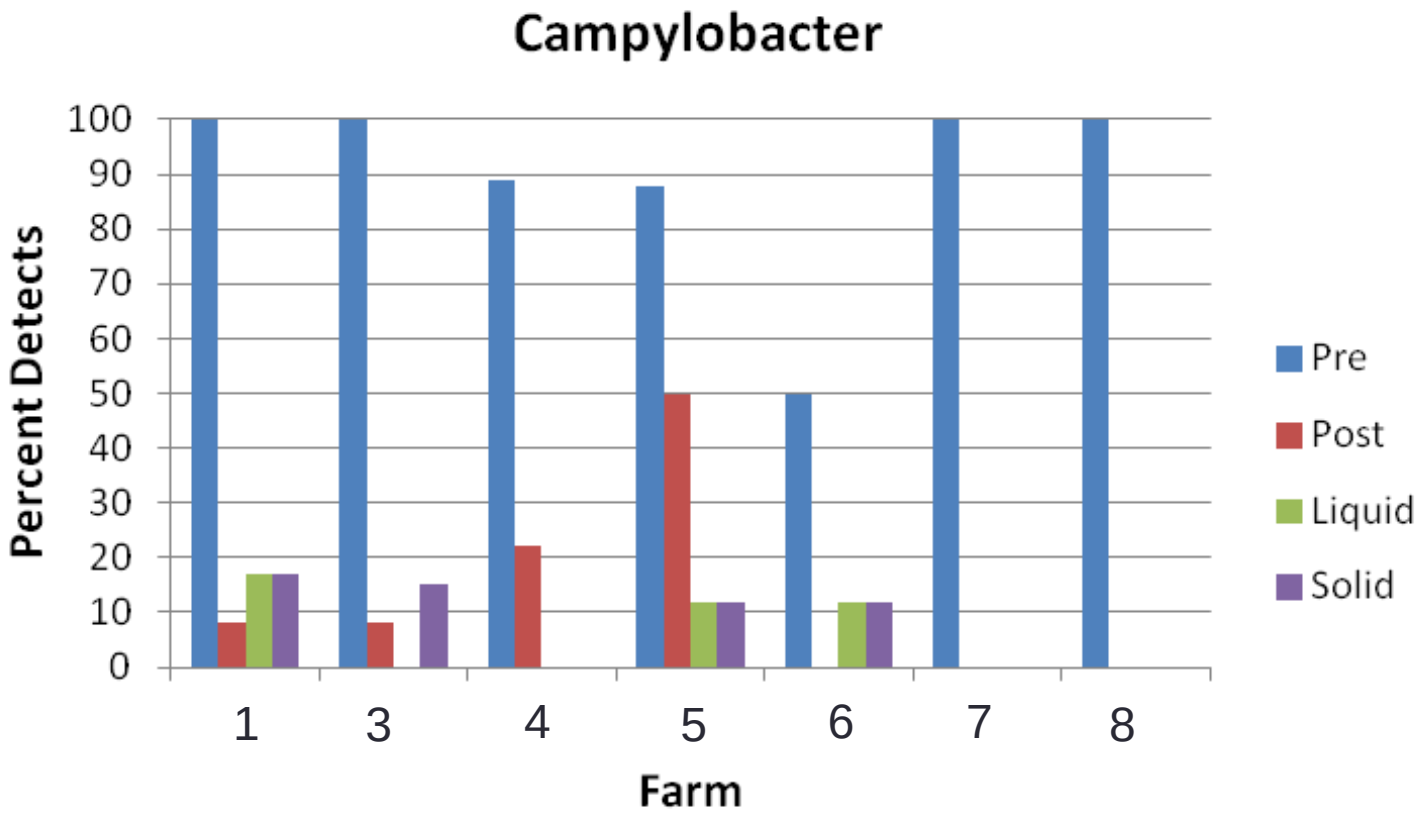
Digester Removal of Polyomavirus over Time



Digester Removal of Coronavirus over Time



Digester Reduction in Percentage Detections



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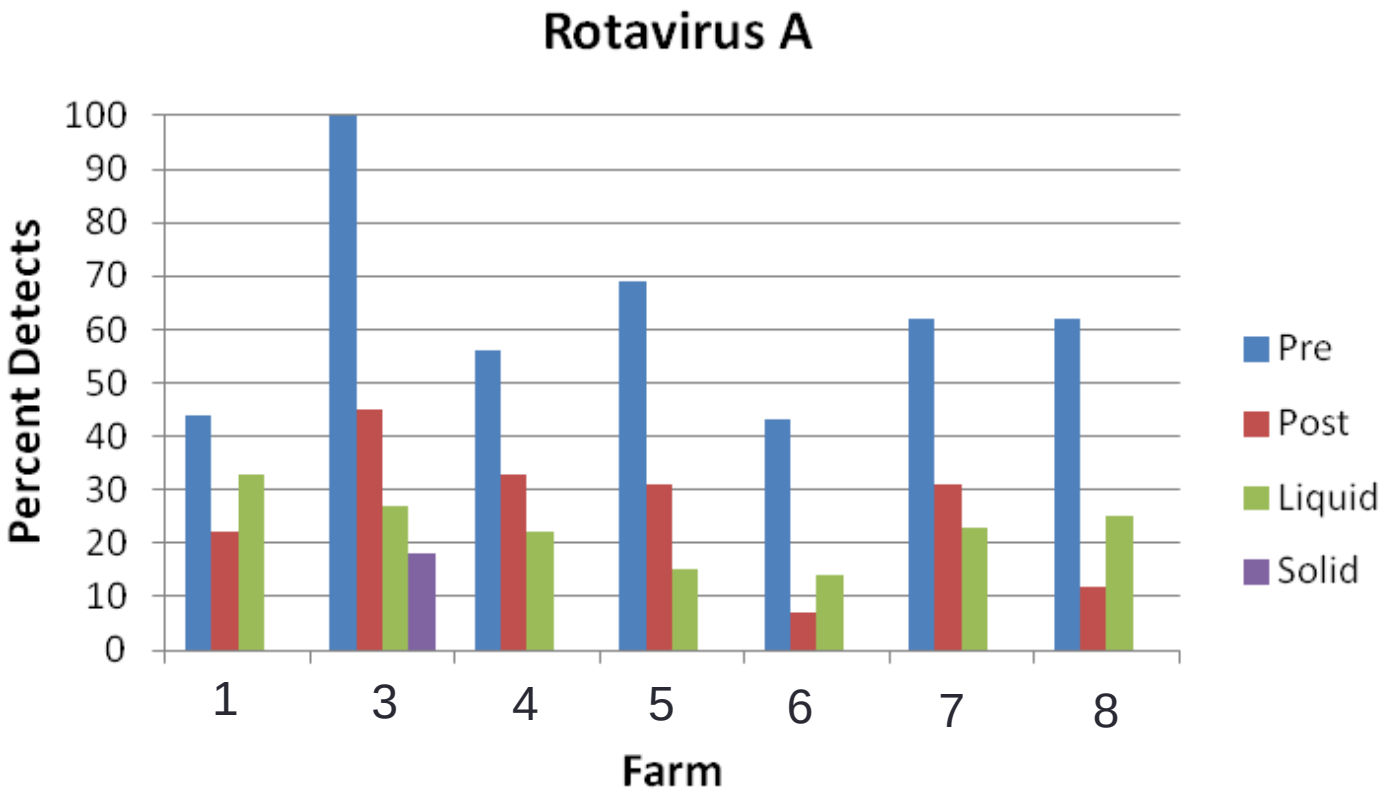
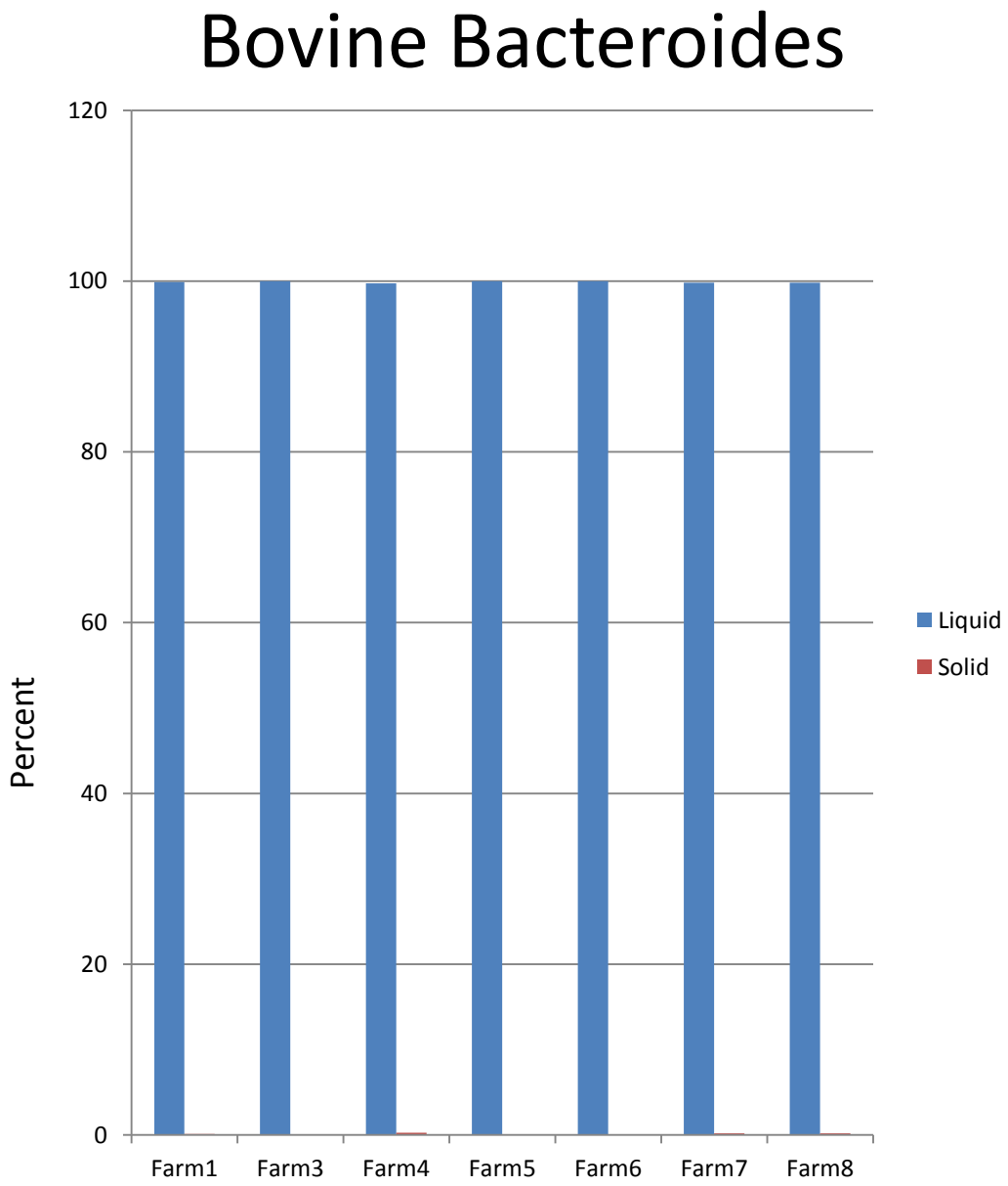


Liquid/Solid Fractionation



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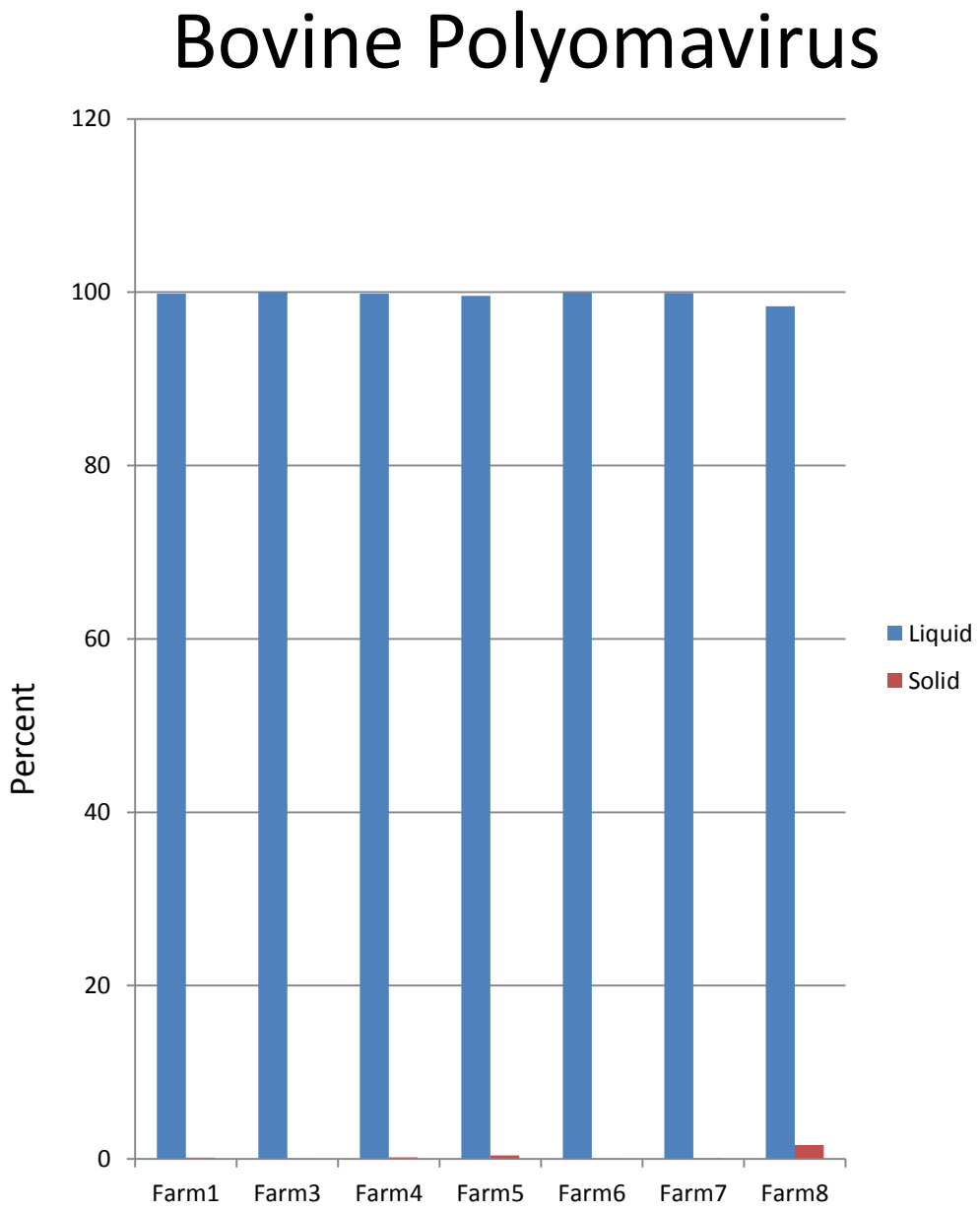
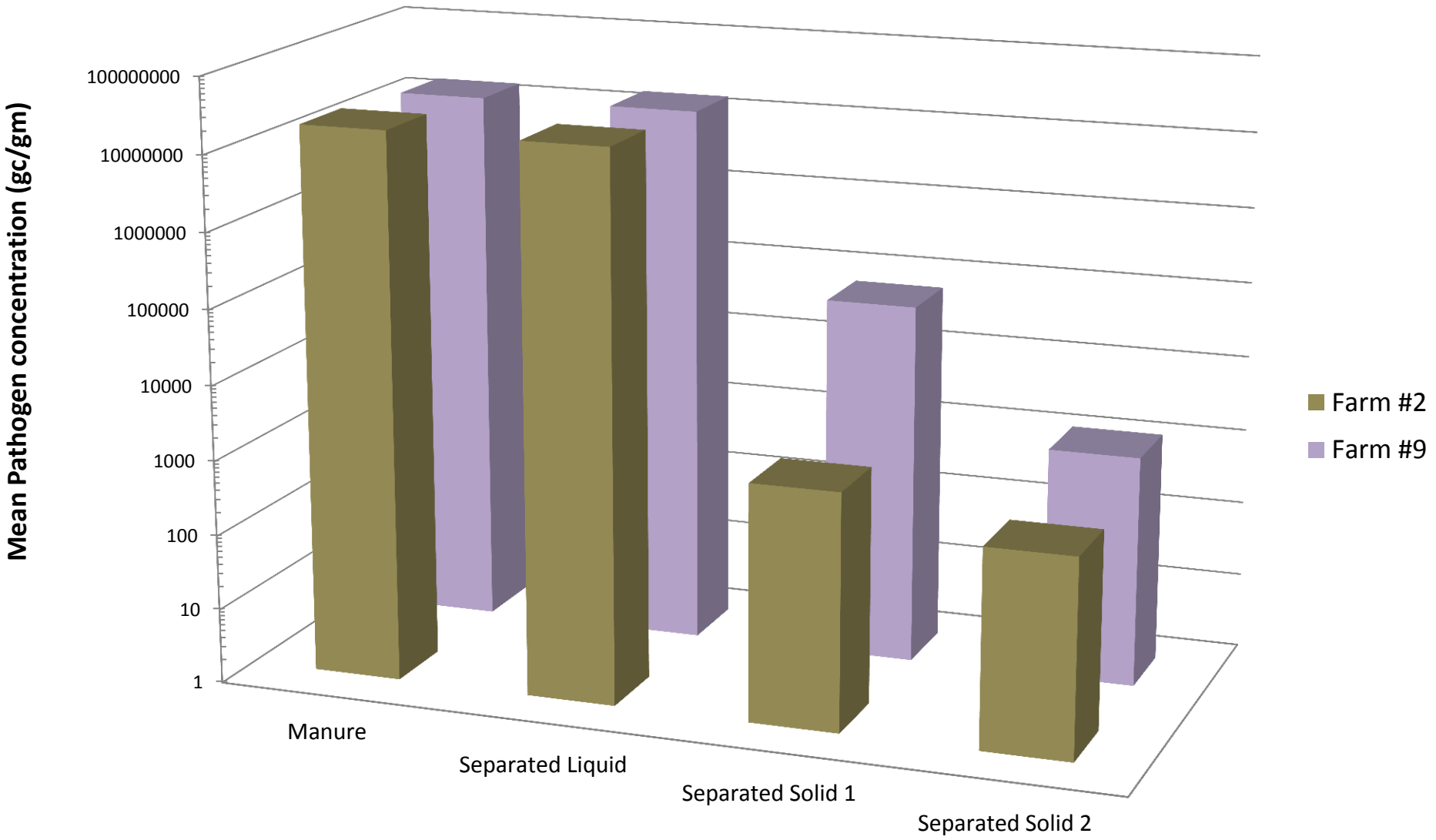
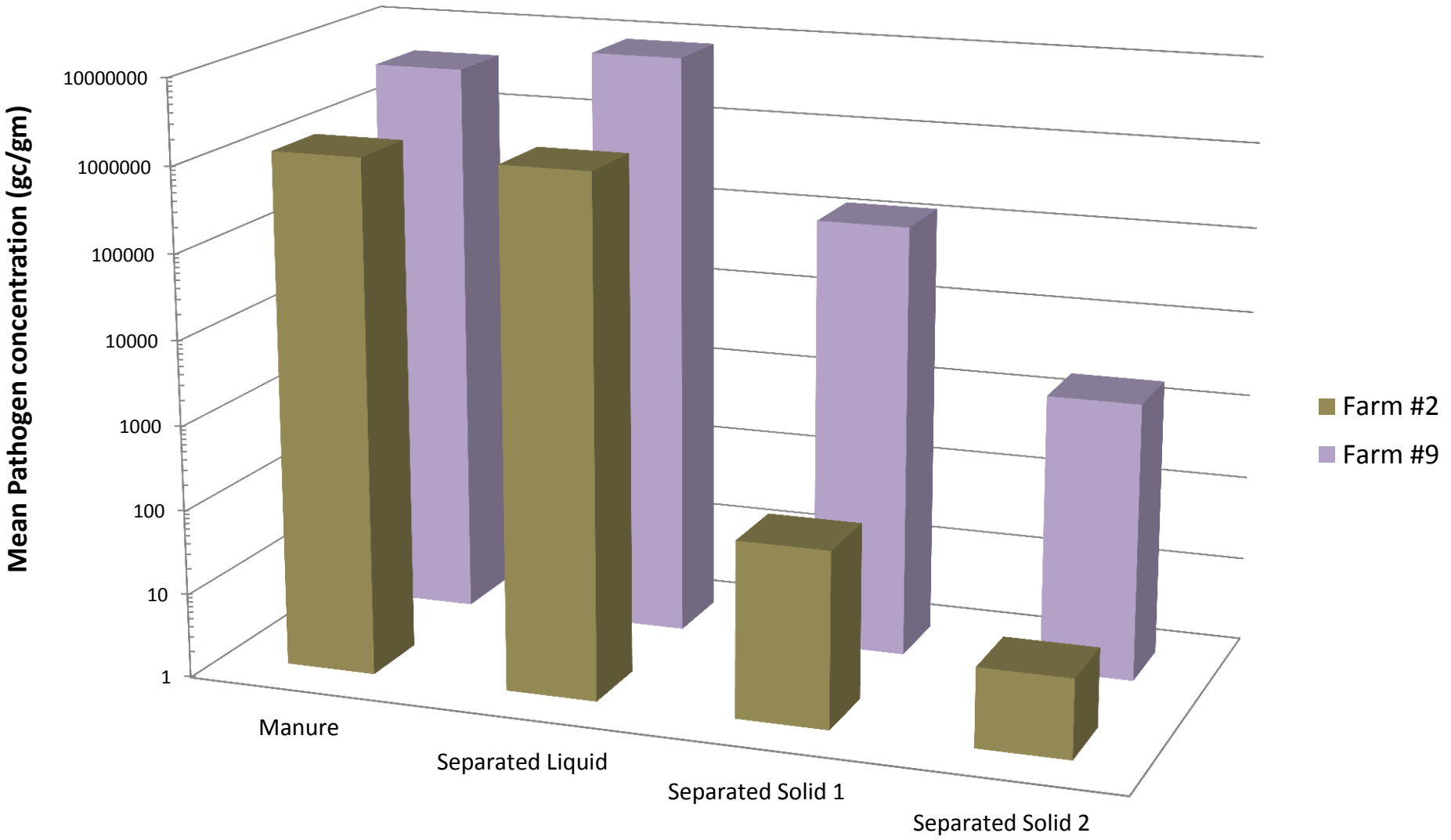


Separated Bedding: Bovine Bacteroides



Separated Bedding: 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?

Findings and perspectives to keep in mind…

- 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.

Questions? Comments?