Survival Kinetics of *Cryptosporidium* oocysts in swine facility wastes of the Southern Piedmont and Coast

Dwight D. Bowman, MS, PhD
Janice L. Liotta
Cornell University

Michael B. Jenkins, PhD
J. Phil Campbell Sr., Natural Resource Conservation Center

Award: 2006-35102-17191

Long-term Goals & Objectives

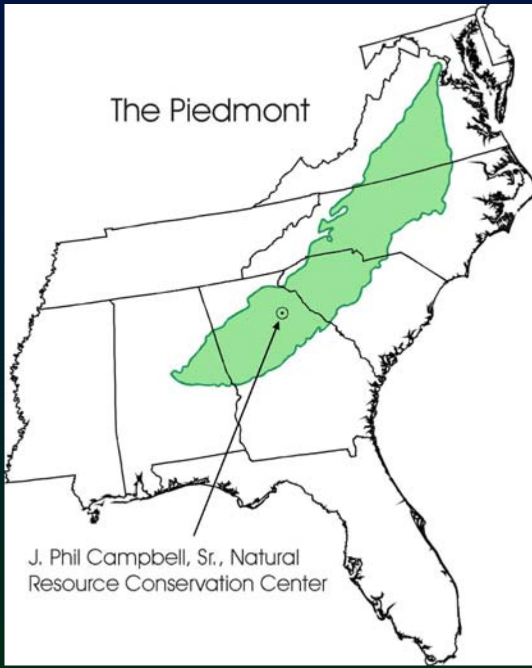
- Minimize the impact of swine rearing facilities on watersheds by abrogating or reducing the number of oocysts leaving the facilities in their waste streams
  - Focusing on facilities in the Piedmont and Coastal Plain Watersheds

Specifically

- In this work, will
  1. Identify and characterize the species and genotypes in swine waste lagoons
  2. Examine inactivation kinetics of oocysts in waste treatment lagoons to ascertain treatment impacts on oocyst viability
  3. Examine the effects of 1° and 2° lagoons on oocyst viability
- Believed that oocysts can be used to monitor how well the processes remove or destroy other pathogens to protect the environment from infectious agents.

Piedmont and Coastal Plain Watersheds

- Between Appalachian Mountains and the Coastal Plain
- Characterized by:
  - Rolling topography
  - Abundant precipitation and surface water resources
  - Landscape of forest, woods, pasture, cropland, creeks, streams and urban sprawl
- Topographic slopes from 2-10%
- Bedrock begins 3 to 30 meters below ground surface
- Soils Cecil and Pacolet sandy loam and sandy clay loam
- Saprolite (partially weathered bed rock) between the bed rock and soil acts as storage and transport media
- Mean annual temperature = 16.3°C; rainfall = 1252 mm



The Coastal Plain

- Extends from the "fall line" separating it from the Piedmont east to the coast
- A flat limestone plain cut by narrow valleys
- Landscape is forests, woods, pasture, cropland, creeks, streams
- More rural than Piedmont
- Soils sandy and low in organic matter
- Mean annual temperature = 16.3°C; rainfall = 1131 mm

Cryptosporidiosis in swine

- Only recently received attention as a separate organisms
  - Almost all infections once thought due to *Cryptosporidium parvum*
- In pigs, infections can be due to several species/genotypes
  - Recently characterized *Cryptosporidium suis*
  - *Cryptosporidium parvum* of cattle
  - A novel swine genotype, *Cryptosporidium* Pig Genotype 2 that appears most related to *Cryptosporidium canis*
- *C. suis*
  - Is not infectious to neonatal nude mice
  - Is poorly if at all infectious to calves
  - *Cryptosporidium suis* reported once from a patient infected with HIV

Cryptosporidiosis in farmed swine

- Poorly characterized relative to prevalence or genotypes
  - In Australia, the prevalence in pigs was 6%
  - In Japan, weaned piglets had a prevalence of 33%
  - In Germany, 1.4% of piglets with diarrhea were found infected
  - In Korea, 10% of porcine fecal samples were positive
  - On a Canadian farm, 100% of 33 piglets all became infected sometime between 21 and 85 days post weaning
    - All isolates of oocysts from these pigs found to be *C. suis*.
- Dr. Fayer, USDA/ARS Beltsville, is currently surveying pigs from birth to market age to differentiate species, genotypes and subgenotypes relative to age (R. Fayer, personal comm.).

Swine and waste management

- Farrow to finish involves four separate operations
  1. Breeding and gestation
  2. Farrowing sows
  3. Nursery (getting piglets ready for finishing)
  4. Finishing (feeding pigs from the nursery to market size of around 240 pounds)
- Each stage requires a separate facility and waste management operations
- Waste management operations usually are a pit below where the swine are housed
- Waste in the underfloor pit is transferred into a lagoon using effluent from the waste lagoon as wash water to drain the pits
  - Solids and liquid are agitated before the waste in the pit is pumped to storage
  - Water is added to the slurry to bring the solids content to less than 4%
- The most common storage lagoon is anaerobic
- The primary lagoon may or may not drain into a secondary lagoon
- Liquid from the lagoon is applied to agricultural fields


Waste lagoons reduce BOD and pathogen loads

- Primary swine waste lagoons substantially reduce concentrations of *Salmonella*, fecal coliforms, *E. coli*, enterococci, and coliphages, but do not reduce concentrations of *C. perfrigens* spores
- In two-stage systems *Salmonella* levels can be further reduced
- To reduce the viability of oocysts of *Cryptosporidium* spp or eggs of *Ascaris suum* present in the lagoons a two-stage lagoons or alternative waste management systems may be needed
- The storing the oocysts of *C. parvum* from cattle in anaerobic pig slurries in England reduced oocysts by 50% after 3 months but oocysts were still over 50% viable
- When applied onto Fescue plots, the number of oocysts declined over 1 to 2 months to undetectable levels, but there was no reduction in oocyst viability during this time

APPROACH

- Processes that effectively inactivate or minimize the transport of oocysts will have a greater capacity to inactivate, remove, or entrap viruses and bacteria present in swine waste
- Because the oocysts of *Cryptosporidium* are likely to be present in most if not all swine lagoons, we believe that they can be used as indicators of the effectiveness of swine waste lagoons in pathogen reduction
- This work will
  - Examine the viability of oocysts present in lagoons
  - Define the effects of the lagooning of swine waste
  - Examine the effects of pretreating swine waste in lagoons on the ability of oocysts to survive after land application.

OBJECTIVE 1

- **Determine the viability of oocysts within swine lagoons and material leaving the lagoons for land application**
  - Oocysts will be collected from the lagoons and purified from the waste material
  - Viability determined by a Dye Permeability Assay
  - Also determine the concentration and prevalence of different species and genotypes of *Cryptosporidium* oocysts present in the swine waste lagoons from the different treatment facilities
    - Thus, will have some indication as to the species and genotypes for which we are obtaining viability data

HYPOTHESIS 1

- The proportion of *C. suis* to *C. parvum* is 90/10, and 5-10% of the recovered oocysts will be viable
  - The majority of the oocysts found will be *C. suis*
    - Although 50% of the oocysts recovered in Australia were *C. parvum*, and all those recovered from pigs in Canada were *C. suis*.
  - The viability of the recovered oocysts will depend on the age of the lagooned sludge.
    - Based on our work on the viability of oocysts in anaerobic cattle waste at 4C, we might suspect that some 80% to 90% of the oocysts would be viable after 300 days
    - However, at the elevated temperatures in the lagoons, we expect markedly higher kinetics of decay
    - Our work in anaerobic digesters showed that all oocysts were inactivated within 10 days if the digesters were held at 37C
  - The rate of inactivation could be quite high in these storage lagoons.

OBJECTIVE 2

- **Determine the effects of lagoon storage on the inactivation kinetics of oocysts**
  - Oocysts placed within the lagoons in sentinel chambers
  - Ascertain the effects of the lagoon process on oocysts and ascertain survival kinetics
  - Primary lagoons typically have standard hydraulic detention of between 6 months to a year
  - Secondary lagoons have shorter detention times (2 to 4 months
- Oocysts placed and recovered at regular intervals

HYPOTHESIS 2

- Detention time in the primary or secondary lagoon will determine oocyst viability
  - Temperature also expected to have a marked effect on oocyst viability
- Therefore, oocysts will be placed in the lagoons using sentinel chambers with the temperature being carefully monitored.

OBJECTIVE 3

- **Determine the inactivation kinetics of oocysts land applied to forage crops after their treatment in swine lagoons**
  - In soil plots in the Piedmont Watershed
  - Four sets of oocysts
    1. Oocysts incubated 3 months in primary lagoon
    2. Oocysts incubated 6 months in primary lagoon
    3. Oocysts from treatment 1 placed in secondary lagoon for 2 months
    4. Oocysts from treatment 2 placed in secondary lagoon for 2 months
  - Oocysts applied to replicate 3 x 3m tilled plots as oocysts in chambers buried 2 cm deep and plots irrigated with remaining oocysts suspension
  - Sampled at 0, 20, 55, and 148 days: chambers and 5 soil samples taken
  - Oocysts extracted from the soil and chambers
  - Viability of oocysts in chambers and soil compared

- During the final year, will attempt to sample soils upon which the waste has been applied from unspiked lagoons with high background oocyst levels
- This will provide an indication as to the levels of oocysts that we can detect on treated soils.

HYPOTHESIS 3

- Lagoon detention times will determine how long oocysts survive after field application
- Oocysts in chambers will behave in the same manner as oocysts applied directly to the soil.

Expected Outcomes

- Role of pigs as a source of oocysts entering watersheds has been relatively neglected compared with the attention that has been given to the examination of cattle as sources
- Work will characterize the problem by determining the types, concentrations, and viabilities of oocysts present in pig waste
- Routine storage in anaerobic lagoons may mean that this problem is markedly reduced compared cattle where the waste is often land applied with minimal storage
- A very good chance that the majority of oocysts are inactivated by the routinely used anaerobic waste lagoon
- The lagoon process will markedly reduce the ability of the oocysts to survive on fields after land application.

This work was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2006-35102-17191