Porcine Respiratory & Enteric Disease Diagnostics:
Utilizing Appropriate Tools for Success

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Swine Production: Opportunities

• The swine industry continues to evolve:
  – Profitable benchmarking
    • Pigs weaned/female/year
    • Pigs born alive/litter
  – Maximizing efficiency
    • Feed efficiency
    • Average daily gain
  – Improving genetics
    • Affects benchmarks and efficiency
  – Innovation
    • Technology, facilities, welfare, records/IT
Swine Production: Challenges

- Animal welfare
- Market competition
- Elevated production costs
- Regulations
- DISEASE

Sustainability
- Society
- Environment
- Economy

Meat Safety
Meat Quality
Health
Genetic Resources
Economy
Working Conditions
Animal Welfare
Societal Conformity
Environmental impact
ISU-VDL: Diagnosing Disease

Top 10 Species Submitted in 2011

- Porcine: 61.6%
- Bovine: 15.6%
- Canine: 7.6%
- Avian: 7.1%
- Equine: 4.5%
- Feline: 1.6%
- Caprine: 0.5%
- Bat: 0.5%
- Ovine: 0.4%

Species Prevalence
Decreased Frequency of Detection

- *Salmonella choleraesuis*
- *Actinobacillus pleuropneumoniae*
- *Bordetella bronchiseptica*

**WHY?**
Many reasons:
- Improved biosecurity
- Production methods
- Vaccination
Increased Frequency of Detection

- *Streptococcus suis*
- *Haemophilus parasuis*
- *Mycoplasma hyorhinis*
- *Actinobacillus suis*
- *PRRSV*
ISU-VDL: Swine Respiratory Disease

Percentage of Porcine Respiratory Cases by Etiology

2011 – 2012 data
Porcine Disease: Pignostics

- Goal: Detecting pathogens of interest
  - Collecting the appropriate samples

- Tissue: lung
- Serum/Blood
- Nasal swab
- Feces
Porcine Oral Fluids

• Oral fluids defined:
  – Saliva
  – Oral mucosal transudate
    • Fluid from blood capillaries

• Oral fluids contain:
  – Pathogens: viruses and bacteria
  – Antibodies

• Oral fluids in humans
  – HIV, Measles, etc.

http://www.pig333.com/what_the_experts_say/oral-fluids-sampling_4761/
Oral Fluids: Pathogens

• Virus detection
  – PRRSV
  – Influenza virus in swine
  – Porcine circovirus type 2

• Bacteria detection
  – *Mycoplasma hyopneumoniae*
  – *Haemophilus parasuis*
  – *Mycoplasma hyorhinis*
Oral Fluids: Diagnostic Tests

• What can we do with oral fluids?
  – PCR: detects the presence of the pathogen
    • Detects small amounts of viruses or bacteria
    • Highly sensitive test
      – PRRSV, Influenza virus in swine, Porcine circovirus type 2
      – M. hyopneumoniae, Haemophilus parasuis, M. hyorhinis
  – ELISA: detects if the pathogen has been there
    • Detects small or large amounts of antibodies in the sample
    • Highly sensitive test
      – PRRSV
      – Influenza antibodies: coming soon
  – Tests require validation before available
    • Make sure the test works
Oral Fluids: Advantages

• Pros, benefits, advantages:
  – Sample collection is rapid
    • Requires about 30 minutes depending on age of pig
  – Sample collection is easy
    • Requires minimal skill
  – Sample collection requires few materials
    • 100% cotton rope (no chemicals)
    • Sterile plastic tube (50 cc)
    • Plastic Ziploc bag (gallon)
    • Scissors

• Nursery pigs
  – 1/2” rope

• Grow/Finish/Adult swine
  – 5/8” rope

Oral Fluids: Sample Collection

- Suspend cotton rope
  - Clean area of pen
    - Away from water, feed, feces
  - Hang at shoulder height
  - Use brackets, gate, chains, etc.

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Oral Fluids: Sample Collection

- **Post-collection**
  - Remove the ‘dry’ portion of the rope
  - Place ‘wet’ portion of rope in plastic bag
  - Squeeze or twist the rope in the bag
    - Collects saliva in the bag and not on the floor
  - Remove a corner of the plastic bag
  - Pour saliva contents into plastic tube

- **Refrigerate or freeze**
  - Approximately 5 cc, preferably more
  - Ship/send as quickly as possible
  - Keep cool and on ice
Oral Fluids: Sample Collection

Oral Fluids: Advantages

- Samples multiple pigs; one specimen
  - “Grab” sample from a population
    - Random number of pigs chew on the rope in a pen
    - Increases the chance of detecting infection
      - Greater representation of the population
    - Reduces need to select particular pigs
- Sampling recommendation
  - 1 rope per 150 pigs
  - 6 ropes per 1200 head finisher
  - Randomly spaced through barn
  - Select individual pens

- Early detection of pathogen
- Sample at 2 week intervals
Oral Fluids: Advantages

- Non-invasive process
  - Eliminates need for:
    - Needles, syringes, hog catcher
    - Less stress, fewer risks
    - Safe, manageable

- Samples are easy to collect
  - Requires minimal skill
  - Requires minimal time
    - 30 minutes, collect, freeze/send

- Less expensive
  - Reduced cost associated with testing multiple animals
  - Materials are inexpensive, stored in bulk
  - More applications are becoming available

- Animal welfare
  - Satisfies producer desire to handle pigs with care
Oral Fluids: Sampling

- Multiple ages
  - Suckling/neonatal piglets
  - Nursery pigs
  - Grower/finisher pigs
  - Dams and boars

Most common

Individual samples
Oral Fluids: Application

• How do we use oral fluids in production systems?
  – Prognostic profiling
    • Monitor pathogens circulating in swine populations
      – What is present at the time of sampling?
        » Viruses and bacteria
        » Other – perhaps in the future
      – Early detection of the pathogens of interest
    • Forecasts the health of the pig population
      – What may occur in the immediate future
        » Infection status
        » Vaccination antibody status
        » Vaccination virus shedding
        » Affects on productivity
Oral Fluids: Application

• Surveillance of a group of pigs
  – Most common use (prognostic profiling)
  – Detection of pathogens: is it present in the sample?
    • At the time of collection
    • Sample collection every two weeks
  – Questions that may be answered with oral fluids:
    • Are pigs infected with a pathogen?
      – Are the pigs presenting with clinical signs
      – PRRSV, PCV, influenza, M. hyopneumoniae
    • Nursery pigs
    • Grow/finish pigs
    • Any age
Oral Fluids: Application

• Surveillance of a group of pigs
  – Questions that may be answered with oral fluids:
    • Are pigs still shedding a pathogen?
      – Post live virus inoculation in a herd
      – Post modified live virus vaccination
      – PRRSV
    • Any age – prior to movement of animals
    • Replacement animals
    • GDU
      – We don’t want to expose a stable population to a virus
Detection of virus: Oral fluid = Serum

Infection/Vaccination

Oral Fluids: Application

• Surveillance of a group of pigs
  – Questions that may be answered with oral fluids:
    • Did pigs produce an antibody response?
      – Detectable antibodies: PRRSV after LVI or exposure
      – Post-vaccination: did pigs receive their vaccine?
      – Prior to movement or exposure to endemic pathogens
    • Replacement animals
    • Any age
Oral Fluids: Application

• Monitoring
  – Checking if swine are negative for pathogens
  – Questions that may be answered with oral fluids:
    • Are pigs negative for a pathogen as represented?
      – Transporting negative pigs?
      – Weaning PRRSV negative pigs?
      – Purchasing negative animals or breeding stock?
      – Negative animals post-isolation?
    • Nursery pigs or any age
    • Replacement animals
    • Boar studs
Oral Fluids: Application

• Vaccination
  – Questions that may be answered with oral fluids:
    • When should I vaccinate?
      – Vaccinate prior to exposure
        » Use oral fluids to help understand when pigs become infected
    • Nursery pigs

• Virus isolation
  • Can I isolate and sequence pathogens in the system?
    – Isolate a virus for sequencing
      » Compare to other viruses previously in the system
      » Use for autogenous vaccine production
  • All ages

Control, elimination, eradication of pathogens
Oral Fluids: Disadvantages

• Cons, pitfalls, disadvantages
  – Small piglets may require training
    • Suckling pigs or piglets nursing dams
    • Not impossible
      – Pigs can be trained to chew on cotton ropes
      – True for any age (small nursery pigs)
      – Entice pigs using a practice rope thrown in pens
      – Flavor practice ropes with sugar solution.
  – Sample quality affects testing
    • Sample inhibitors that may affect the test
      – Dirt, feces, enzymes
    • Viral/bacterial degradation
    • Difficulty isolating and sequencing pathogens
Oral Fluids: Disadvantages

• Cons, pitfalls, disadvantages
  – Environmental contamination
    • Pathogens that remain in the environment can be detected
      – Clean and disinfect between groups of pigs
      – This is only true for pathogens that persist in the environment
        » Porcine circovirus
      – Follow-up testing may be necessary
  – Antibodies in oral fluid from plasma protein in feed
    • Antibody tests will detect antibodies in feed
      – Only if plasma protein products are utilized in the feed
        » Nursery pig feed most common
      – The antibodies are detected in the oral fluid
        » False positive results
      – Remove plasma protein and pigs are negative
        » Typically 24 hour time frame
Oral Fluids: Disadvantages

- Cons, pitfalls, disadvantages
  - Detection but poor isolation of pathogen
    - Suboptimal success at isolation of pathogens
      - May need to use another sample type for autogenous vaccine production
  - Detection but not a diagnosis
    - Detection does not indicate a diagnosis of disease
      - Diagnostic samples (tissue, blood, etc.)
    - Prognostic profiling: herd level versus animal level

Use your veterinarian
Oral Fluid: Implications

• Do not pool oral fluids
  – Oral fluids are already collected from multiple animals
  – Do not combine samples into one composite

• Purchase cotton rope through the internet
  – Not always available at local stores
  – Don’t use synthetic material

• Cotton rope (1/2” to 5/8”)

• Train pigs when reluctant to approach the rope

• Collect samples during morning hours
  – Pigs are typically more active
Oral Fluid: Implications

- Hang shoulder height
  - Keep away from the water supply and feeder
  - Don’t use rope that is too long
- 20-30 minutes in the pen is sufficient
- Squeeze rope in plastic bag or bootie
  - Pour into a suitable tube for submission
- 5 ml of oral fluid is ideal
- Freeze or chill
- Do not re-use old ropes
- Don’t swab the pig’s mouth
Percentage of Porcine Enteric Cases by Etiology

Frequency of diagnosis at the ISU-VDL: All ages

2011 - 2012

- Rotavirus
- Salmonella spp.
- Hemolytic E. coli
- Lawsonia
- E. coli
- Idiopathic enteritis
- TGE
- Coccidia
- Salmonella group B
- Bact.
- C. difficile
- Brachyspira spp.
- C. perfringens
- PCV
- viral
Frequency of Enteric Disease Diagnosis by Age

**Neonate: 0 - 3 Weeks Old**

- Rotavirus: 50%
- Bacterial: 0%
- E. coli: 10%
- C. difficile: 20%
- Salmonella: 30%
- C. perfringens: 40%
- Coccidiosis: 50%
- Viral: 0%
- TGE: 0%
- Ileitis: 0%

**Nursery: 3 – 8 Weeks Old**

- Rotavirus: 50%
- E. coli: 40%
- Salmonella: 30%
- Coccidiosis: 20%
- Viral: 10%
- Bacterial: 0%
- TGE: 0%
- Ileitis: 0%
- Brachyspira: 0%
Frequency of Enteric Disease Diagnosis by Age

Grow/Finish Swine: 8 - 26 Weeks Old

- Enteritis
- Salmonella
- E. coli
- TGE
- Brachyspira
- Bacterial
- Rotavirus
- Hem. bowel
- Coccidia
- PCV2
Postweaning Enteric Disease: Samples

- **Antemortem**
  - Feces or fecal swabs
  - Collection
    - Representative pigs or fecal samples
    - Feces from 10-15 affected pigs
    - Fecal swabs from 15-30 affected pigs
    - Pools feces or swabs up to 5 samples
Postweaning Enteric Disease: Samples

• Fecal Swabs
  – Synthetic swab (dacron or rayon) plastic shaft
  – Keep cool, prevent drying during transit
    • Viral/bacterial transport media, PBS, saline
    • Avoid agar gel-type media
    • Avoid calcium alginate-tipped swabs
      – May inhibit testing ability
      – Ask your veterinarian
  – Starswab II, Copan E-swab
Postweaning Enteric Disease: Samples

• Postmortem Samples:
  – Select 3 – 4 recently sick pigs
    • Euthanize appropriate animals with approved methods
    • Select animals representative of the problem
    • Submit tissues from more than 1 acutely affected animal

• Always submit both small intestine and colon
  – Fresh: chilled, submit on ice

• Feces
  – Collect from the colon not the small intestine
Enteric Disease: Porcine Rotavirus

- Increased frequency of detection
  - Test sensitivity, increased testing, other?
Rotavirus: Serotype Age Distribution

- **PCR: Serotypes A, B and C**
- **Rotavirus detection by age:**
  - Rotavirus Type A:
    - More frequently detected 3 – 6 weeks
  - Rotavirus Type C
    - More frequently detected < 1 week of age

Group A
- 39% < 1 week
- 29% 1-3 weeks
- 25% 3-6 weeks
- 7% > 6 weeks

Group B
- 31% < 1 week
- 16% 1-3 weeks
- 31% 3-6 weeks
- 22% > 6 weeks

Group C
- 56% < 1 week
- 25% 1-3 weeks
- 9% 3-6 weeks
- 10% > 6 weeks
Enteric Disease: TGE

- Less common in farrowing and nurseries
Finisher Pigs: Ileitis

- Grow/finish pigs
  - Easily over or under estimated
  - Feces and fecal swabs
  - Select tissues for lesion evaluation
    - Small intestine
    - Colon

![Ileitis Graph]

**Ileitis**

- Total Number of Cases
- Year

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IOWA STATE UNIVERSITY
Veterinary Diagnostic Laboratory
Enteric Disease: Brachyspira

- *Brachyspira*-associated colitis
- “Bloody Scours”
  - Colon, feces, fecal swabs
  - *Brachyspira*
    - *hyodysenteriae*
    - *Hampsonii*
      - New species
    - *pilosicoli*
    - *murdochii*
    - *intermedia*
    - *Innocens*

![Bar chart showing the total number of cases diagnosed from 2003 to 2012. The chart indicates a significant increase in cases from 2010 onwards.]
Enteric Disease: Bacteria

• Hemolytic *E. coli*
  – Fimbrial genes
    • K88, F18, 987p, F41
  – Toxin genes
    • Stb, Sta, LT, Stx2e

• *C. perfringens*
  – Genotypes
    • A, B, C, D and E
  – Toxin genes
    • Beta-2 and Enterotoxin
Enteric Disease: Other

- **Salmonella**
  - Bacterial culture and lesions

- **Coccidia**
  - Fecal exam
  - Intestinal lesions
Conclusion

• Work with your herd veterinarian
• Involve the veterinarian early in the disease process
• Select pigs that recently became sick for sampling
• Oral fluids
  – Easy to collect and useful diagnostic tool
  – Interpret results with your veterinarian
• Enteric disease
  – Fecal samples
  – Fresh tissue from recently sick pigs
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