

Comparison of Swabs and Tissue for Detection and Characterization of *Escherichia coli* in Clinical Post-weaning Diarrhea Cases

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Summary and Implications

Submitting tissue samples via mail may present challenges to practitioners with respect to public health, public perception, and regulatory restrictions. Considering these challenges of tissue sampling, the objective of this pilot study was to evaluate alternative sampling techniques for the detection and characterization of *Escherichia coli* (*E. coli*) in field cases of diarrhea in weaned pigs. Rectal swabs offer a practical alternative to tissue sampling because they potentially decrease the need for tissue sampling and allow an increased sample size in a more cost effective manner. Rectal swab samples were compared to intestinal tissue samples from the same pig to compare the frequency of *E. coli* isolation, and agreement of both antibiograms and genotyping for pilus and toxin genes. Diagnostic results were evaluated for agreement at the pig and farm level. *E. coli* was isolated from all cases using both rectal swabs and intestinal tissue. The genotyping results from the rectal swab and the intestinal tissue did not agree at the pig level in 64% of the cases. This suggests that multiple samples are required to characterize the *E. coli* population in field cases, and if both results are considered, we are more likely to choose an effective treatment to cover the entire population. Rectal swabs and tissue samples both have individual advantages and disadvantages to the practitioner. Tissue samples give the practitioner the ability to necropsy the pig and therefore view systematic lesions and other pathogens. Rectal swabbing may provide an opportunity for practitioners to submit a greater number of samples per farm to better characterize the *E. coli* population without euthanizing additional pigs but may not replace tissue derived diagnostics entirely. When facing a difficult *E. coli* challenge or poorly represented and identified populations, we can cost effectively increase the sample size by adding rectal swabbing to current diagnostic tools.

Introduction

E. coli is an organism that is always present in the digestive tract, but is not an issue unless both pili and toxin genes are present. With both genes present, *E. coli* then

causes diarrhea and other problems for practitioners. Diagnostic tests determine the genotype and antibiotic sensitivity of the *E. coli* present in the individual pig. Submitting tissue samples via mail for intestinal tissue *E. coli* diagnostics may present challenges to practitioners with respect to public health, public perception, and regulatory restrictions. The credibility of diagnostic data needed for clinical treatment decisions may be put at risk by sample handling, holding temperatures, and transportation hazards. Additionally, the cost of shipping tissue weight may limit the number of samples submitted. Post mortem samples that require pigs to be sacrificed impose higher costs to producers than antemortem samples and potentially impact animal welfare. Rectal swabs offer a practical alternative to tissue sampling because they potentially decrease the need for tissue sampling and allow an increased sample size in a more cost effective manner. Considering these challenges of tissue sampling, the objective of this pilot study was to evaluate alternative sampling techniques for the detection and characterization of *Escherichia coli* (*E. coli*) in field cases of diarrhea in weaned pigs.

Materials and Methods

Treatments: Treatment one; Intestinal tissue was the ‘gold standard’ or control for this study. This sample type is the most common sample taken by practitioners and submitted for *E. coli* detection and characterization. Treatment two: Rectal swab samples were compared to intestinal tissue samples from the same pig to compare the frequency of *E. coli* isolation, agreement of antibiograms and genotyping for pilus and toxin genes. This allowed one pig to serve as its own control.

Case definition: A case was defined as one untreated post-weaning pig that exhibited clinical diarrhea and perineal hyperemia. Cases came from a flow with a suspected history of *E. coli*, with the practitioner suggesting pigs that would usually be sampled for *E. coli*.

Animals: A total of 15 pigs from four sites were sampled. These sites were conventional confinement facilities in the Midwest. Sex, genetics, and other differences between individual pigs were not evaluated being as the pigs served as their own control in this study.

Samples: Two rectal swab samples, two intestinal swab samples, and a tissue sample were collected from each pig. Both a rectal swab and tissue from each pig were cultured

for *E. coli*, and fixed tissues were evaluated for concurrent lesions.

Assays: When *E. coli* was cultured, antimicrobial bacterial sensitivity and multiplex polymerase chain reaction testing (PCR) for toxin and pilus genes were conducted. The standard Iowa State University Veterinary Diagnostic Laboratory food animal antibiogram was used to determine the antimicrobial sensitivity but eliminate classes that were not expected to have any effective activity on *E. coli*. Antimicrobial sensitivity evaluation was focused on five antimicrobials most relevant to clinical settings: ceftiofur, gentamycin, neomycin, and trimethoprim/sulphamethoxazole. Diagnostic results were evaluated for agreement of genotyping and antibiotic sensitivity at the pig and farm level.

Measures:

Genotype- The presence or absence of F18 and K88 pili genes and STa, STb, and LT toxin genes was determined. **Antibiotic sensitivity-** The sensitivity of *E. coli* to ceftiofur, gentamicin, neomycin, and trimethoprim/sulphamethoxazole, antibiotics was determined, **Agreement-** Agreement was based upon comparing the genotype and antibiotic sensitivity results from the diagnostics, and was only found if all results from the two tests matched.

Results and Discussion

E. coli was isolated from all cases using both rectal swabs and intestinal tissue with the results presented descriptively in Table 1. Rectal swabs yielded *E. coli* with both toxin and pili genes in pigs with clinical signs as opposed to normal flora. One rectal swab yielded an *E. coli* isolate with STa, STb, and LT toxin genes, in addition to both F18 and K88 pilus genes. A study reported in Diseases of Swine by Fairbrother and Gyles (1985) reported one individual case carrying up to 25 strains of *E. coli*, in respect to O serogrouping, genotyping, and antibiotic susceptibility. Both of these studies are excellent demonstrations of the variety of pathogenic *E. coli* genotypes in a single pig population.

The antibiotic sensitivity and genotyping results from the rectal swab and the intestinal tissue did not agree at the pig level in 64% of the cases. At the site level on three of four farms, *E. coli* genotypes were identified among the rectal swabs that were not represented in the intestinal tissues of the same site and vice versa. On those farms, there were also different antibiograms identified among the rectal swabs compared to tissues samples and vice versa. On these four sites, rectal swabbing did not produce the same

diagnostic information as tissue samples, and may have led to a different treatment strategy. On three of the four farms, neither tissue isolates alone nor rectal swabs alone detected all of the pathogenic genotypes on the site. Basing treatment strategies solely on rectal swabs alone or tissue samples alone may not effectively treat the *E. coli* challenge. This suggests that multiple samples are required to characterize the *E. coli* population in field cases, and if both results are considered, we are more likely to choose an effective treatment to cover the entire population.

Advantages and Disadvantages of Sample Site and Methods

Rectal swabs and tissue samples both have individual advantages and disadvantages to the practitioner. Tissue samples give the practitioner the ability to necropsy the pig and therefore view systematic lesions and other pathogens. *E. coli* is a ubiquitous organism in that presence does not always equal disease, so the histopath gives an opportunity to verify diagnosis. Though swabs do not offer those abilities, they do allow for sampling of healthy pigs or acute cases in which practitioners may be reluctant to euthanize. Rectal swabs allow survival of the pig, and therefore save the cost of the entire pig. Rectal swabs also allow an increased sample size, and running more antibiotic sensitivity tests can be justified because of costs saved otherwise from not euthanizing or shipping. A better idea of the overall antibiotic susceptibility of the *E. coli* population in the entire herd is given, justifying the use of antibacterials. Rectal swabbing may provide an opportunity for practitioners to submit a greater number of samples per farm to better characterize the *E. coli* population without euthanizing additional pigs but may not replace tissue derived diagnostics entirely. This clinical tool may be used as a supplemental diagnostic method in addition to tissue submission. When facing a difficult *E. coli* challenge or poorly represented and identified populations, we can cost effectively increase the sample size by adding rectal swabbing to current diagnostic tools.

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Table 1. *E. coli* swab results from 15 nursery pigs taken from 4 conventional confinement facilities in Midwest.

Pig	Sample	Antibiotic Sensitivity ¹	Genotype	Agreement
Farm I				
A	Rectal	Gent	All negative	NO
	Tissue	Trim	STb, K88	
B	Rectal	Gent	All negative	NO
	Tissue	Trim	STb, K88	
C	Rectal	Ceft, Gent, Neom, Trim	All negative	NO
	Tissue	Ceft, Gent, Trim	STb, LT, K88	
D	Rectal	Ceft, Gent, Neom, Trim	All negative	NO
	Tissue	Ceft, Gent, Neom, Trim	STb, Sta	
E	Rectal	Ceft, Gent, Neom, Trim	STb, K88	YES
	Tissue	Ceft, Gent, Neom, Trim	STb, K88	
Farm II				
A	Rectal	Gent, Neom, Trim	STb, Sta, F18	NO
	Tissue	Ceft, Gent, Trim	Sta, K99	
B	Rectal	Ceft, Gent, Neom, Trim	STb, Sta, K88	NO
	Tissue	Ceft, Gent, Trim	Sta, K99,	
C	Rectal	Ceft, Gent, Neom, Trim	STb, Sta, LT, F18, K88	NO
	Tissue	Ceft, Gent, Neom, Trim	STb, Sta, F18	
Farm III				
A	Rectal			2
	Tissue	Ceft, Trim	STb, LT, K88	
B	Rectal	Ceft, Gent, Neom, Trim	STb, LT, K88	YES
	Tissue	Ceft, Gent, Neom, Trim	STb, LT, K88	
C	Rectal	Ceft, Gent, Neom, Trim	K88	YES
	Tissue	Ceft, Gent, Neom, Trim	K88	
D	Rectal	Ceft, Gent, Trim	F18	NO
	Tissue	Ceft, Trim	STb, Sta	
Farm IV				
A	Rectal	Ceft, Gent, Trim	F18	YES
	Tissue	Ceft, Gent, Trim	F18	
B	Rectal	Ceft, Gent, Trim	F18	NO
	Tissue	Ceft, Trim	F18	
C	Rectal	Ceft, Gent, Trim	F18	YES
	Tissue	Ceft, Gent, Trim	F18	

¹ Ceft, Ceftriaxone; Gent, Gentamicin; Neom, Neomycin; Trim, Trimethoprim/Sulphamethoxazole

² Results lost to follow up testing

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