Broadening the Scope:
Non-Traditional PathKinex Sampling

PathKinex is an extremely versatile tool that can be applied to samples from multiple tissue sites

PathKinex samples are traditionally sourced from rectal swabs, which have proven effective at detailing pathogen gene marker information from animal GI tracts. Thousands of samples have been collected and catalogued this way, giving us a robust database for comparison. However, in some cases, pathogens are concentrated in tissues outside of the digestive system, presenting the opportunity to investigate these different areas. When these situations arise, we have used our expertise to develop new PathKinex sampling approaches that help reveal pathogenic coinfections in non-traditional tissues[1].

Non-Traditional PathKinex Sampling Approaches

Footpad Swabs

Footpad tissue swabs have been utilized to study pododermatitis in poultry, which causes necrotic lesions on the feet of broilers (1). In this unique PathKinex application, *Enterococcus faecalis* and *Enterococcus cecorum* were found to be positively correlated with severe lesion formation. Genes associated with avian pathogenic *E. coli* and enteroaggregative *E. coli* were also detected in higher levels in footpads with severe lesions. These results suggest that there may be an interplay of multiple pathogens, including *E. faecalis, E. cecorum,* and *E. coli,* that each contribute to the establishment or aggravation of pododermatitis foot lesions. These data provide useful clues in identifying pathogen targets for potential interventions aimed at mitigating lesion formation and promoting healing.

Lesion Score	0 (none)	1 (Moderate)	2 (Severe)
E. cecorum gene copies (Log10)	0.92	1.64	1.84
E. faecalis gene copies (Log10)	0.31	1.04	1.40

Nasal Swabs

Nasal swabs have been collected to uncover microbial pathogen diversity in a swine nursery with known *Streptococcus suis*-associated disease problems. PathKinex results revealed that genes for *S. suis* were detected in all swabs; however, nasal swabs from sick pigs yielded a higher gene quantity compared to healthy pigs. When in the upper respiratory tract, *S. suis* may not cause harm on its own, but specific serotypes can develop pathogenicity and coinfect alongside other bacterial or viral pathogens (2). Interestingly, gene markers for *Pasteurella multocida* were also detected at a significantly higher amount in the sick nasal samples, suggesting a potential coinfection. *P. multocida* is an opportunistic secondary invader associated with respiratory infections and pneumonia (3).

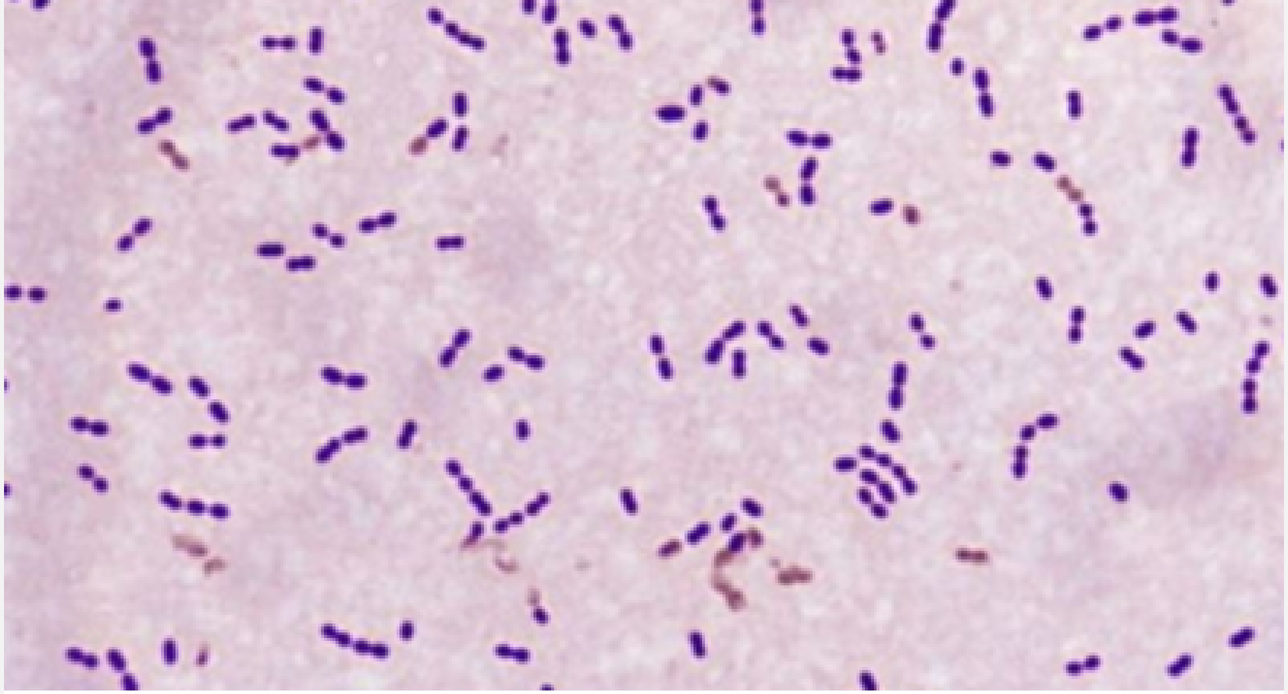

Figure 2. Streptococcus suis gram stain

Intestinal Swabs

In this case, we studied a population of cows that had experienced a recent increase in clinical signs of hemorrhagic bowel syndrome (HBS). HBS is a fatal disease characterized by severe hemorrhage in the small intestine and its pathogenesis is unknown, though prior research has identified *Clostridium perfringens* as an associated microorganism (4). As part of this study, we took swabs from the postmortem intestinal section of one HBS symptom-presenting cow. Culturing and molecular analysis of the DNA isolated from the intestinal swabs confirmed that there were high levels of both *E. coli* and *C. perfringens* present. Further investigation revealed the detection of *Clostridium sordellii*, a highly toxic organism associated with enterotoxemia and sudden death syndrome (5).

While HBS pathogenesis is not yet fully understood, the intestinal swabs yielded pathogens that have the potential to be involved. It is possible that the colonization of *E. coli* creates an intestinal environment conducive to coinfections, including from *C. perfringens*, which in turn accelerates the disease. (The *C. sordellii* detection, while important to acknowledge, must be viewed with caution, as the sample was taken postmortem and this organism can invade and proliferate rapidly after death.)

Intestinal Section Plate Counts and Toxin Gene Quantities

Microorganism	Plate Count (Log_{10} CFU/g)	Toxin Gene Marker	Gene Copies (Log_{10})
E. coli	*6.69*	*EAST1*	*3.6*
Clostridium spp.	*5.72*	*cpa*	*5.0*

Non-Traditional Sampling Takeaways

Each of these examples demonstrates the versatility of PathKinex and highlights the potential of this tool for the investigation of complex diseases in commercial conditions. The microbiome changes rapidly across different environments in the animal body, and in certain situations it can be beneficial to analyze these alternative locations.

Through PathKinex, we have learned that there are often multiple pathogens involved in disease development. Each of the above studies, though performed in different tissues, are no exception. PathKinex results from non-traditional locations can help reveal what those pathogens are and indicate what direct interventions, including *Bacillus*-based DFM products, may be effective at inhibiting pathogen growth and restoring population health.

Discussion Question

For customer-facing Seismic users, consider reviewing the Coinfection Session from the last global sales meeting.
Seismic>Collections>Technical Subjects>GSM 23 – Microbial Cross Species Insights

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Do you have recommendations on additional tissues or diseases that would be valuable to investigate using PathKinex?

Respond to MDG

Is there a topic you'd like to learn more about in a future newsletter? We enjoy hearing from you! We welcome your questions, comments, and suggestions on PathKinex updates. Please contact us at AnimalAg@mdgbio.com

References

1. Shepherd, E. M., & Fairchild, B. D. (2010). Footpad dermatitis in poultry. *Poultry science*, *89*(10), 2043–2051.

2. Estrada, A. A., Gottschalk, M., Rossow, S., Rendahl, A., Gebhart, C., & Marthaler, D. G. (2019). Serotype and Genotype (Multilocus Sequence Type) of Streptococcus suis Isolates from the United States Serve as Predictors of Pathotype. *Journal of clinical microbiology*, *57*(9), e00377-19.

3. Kim, J., Kim, J. W., Oh, S. I., So, B., Kim, W. I., & Kim, H. Y. (2019). Characterisation of Pasteurella multocida isolates from pigs with pneumonia in Korea. *BMC veterinary research*, *15*(1), 119.

4. Dennison, A. C., VanMetre, D. C., Callan, R. J., Dinsmore, P., Mason, G. L., & Ellis, R. P. (2002). Hemorrhagic bowel syndrome in dairy cattle: 22 cases (1997–2000). *Journal of the American Veterinary Medical Association*, *221*(5), 686-689.

5. Vermunt J.J, Malmo J., and Parkinson T.J. 2010, Causes of Sudden Death, Chapter 23 in Parkinson TJ, Vermunt JJ and Malmo J. Diseases of Cattle in Australasia. A comprehensive textbook. New Zealand Veterinary Association Foundation for Continuing Education, Wellington, p 792.

[1]Testing must be completed to verify the reliability and efficacy of new sampling protocols before a new approach is put into practice.

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