INTRODUCTION

• No clear link stating flies transmit pathogens via ingestion of fecal matter.
• Most analysis are done via molecular biology techniques such as DNA sequencing or bacterial titers
• Cumbersome
• No compounds found in literature that tie fly consumption of feces to pathogen transmission

METHODS

• Flies were fed based on control group
• Flies were killed, dissected, and DNA extractions were performed
• 50 µL aliquot of organic layer from DNA extraction was evaporated under nitrogen
• Resuspend in 50 µL of 1:1 methanol:water solution
• Vortex for 10 min
• Separate using Agilent 1100 HPLC system
• Identification on Thermo Fisher LTQ XL™ Linear Ion Trap Quadrupole

RESULTS

Urobilin and urobilinogen were detected at a retention time of 6.5 min and 8.2 min respectively only in flies that fed on feces

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from Feces in Fly Guts

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ULTS

CONCLUSIONS

Conclusions:
• LC MS/MS is a good qualitative test to detect fecal urobilinoids
• No false positives or negatives have been observed
• According to the Kruskal-Wallis test, there was a significant effects of treatments at 6 min (chi-squared = 72.8, df = 3, P < 0.0001) and 8 min (chi-squared = 78.3, df = 3, P < 0.0001)

Potential Problems:
• Many factors can impact the urobilinoid signal intensity
• Feces consumed by the fly
• Exposure of samples to light and air
• Gender of the fly
• Overall instrument variation
• Difficult to get "pure" urobilin standards
• Difficult to fully resolve urobilinoid isomers
• Second peak at ~8.2 min helped with identification of these
• High variation among positive “control” samples
• Standard was used to help with this

Future Directions:
• Apply method using multiple species of filth flies and other coprophagous insects
• Combine the method with microbial culturing and sequencing methods
• Combine the method with vertebrae sequencing methods to identify the source of pathogens

REFERENCES

ACKNOWLEDGEMENTS
We would like to thank the Indiana University-Purdue University Indianapolis Department of Chemistry and Biology for allowing us to perform these experiments