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Novel, Sensitive, and Specific Hookworm Diagnosis From Urine Samples

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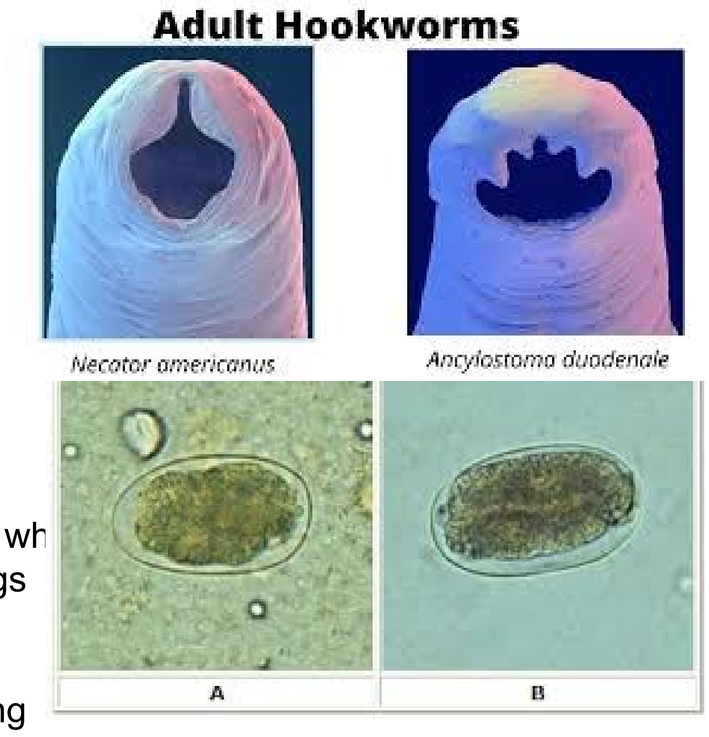
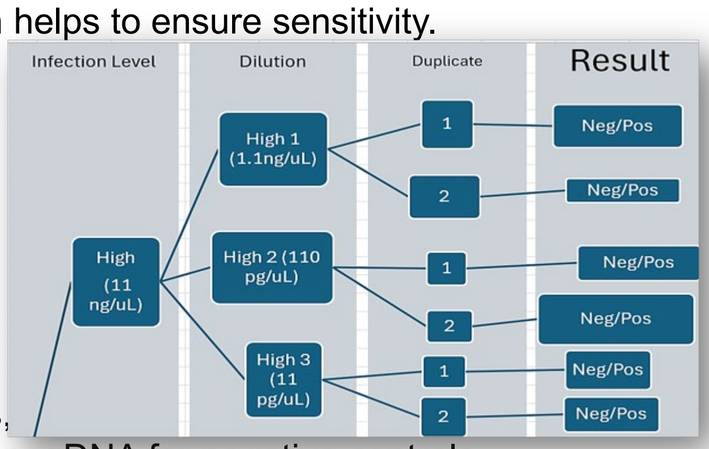
INTRODUCTION & AIM

- **Hookworm** infects nearly 500 million people worldwide.
- The two main human-infecting species are ***Ancylostoma duodenale*** and ***Necator americanus***.
- Hookworm is a neglected tropical parasitic disease, impacting tropical and lower-middle-income countries.
- Hookworm can cause development complications in young children by feeding on the blood from their intestinal wall, which leads to severe anemia.
- Current method of diagnosing Hookworm is ***Kato Katz (KK)***, wh involves detecting parasite eggs in stool.
- *KK* is non-specific, insensitive, and time-consuming and cannot distinguish species based on egg morphologies.
- The **project aims** to demonstrate the feasibility of detecting species-specific, cell-free hookworm DNA from urine samples using PCR.
- This would facilitate mass drug administration (MDA) and reduce hookworm cases worldwide.

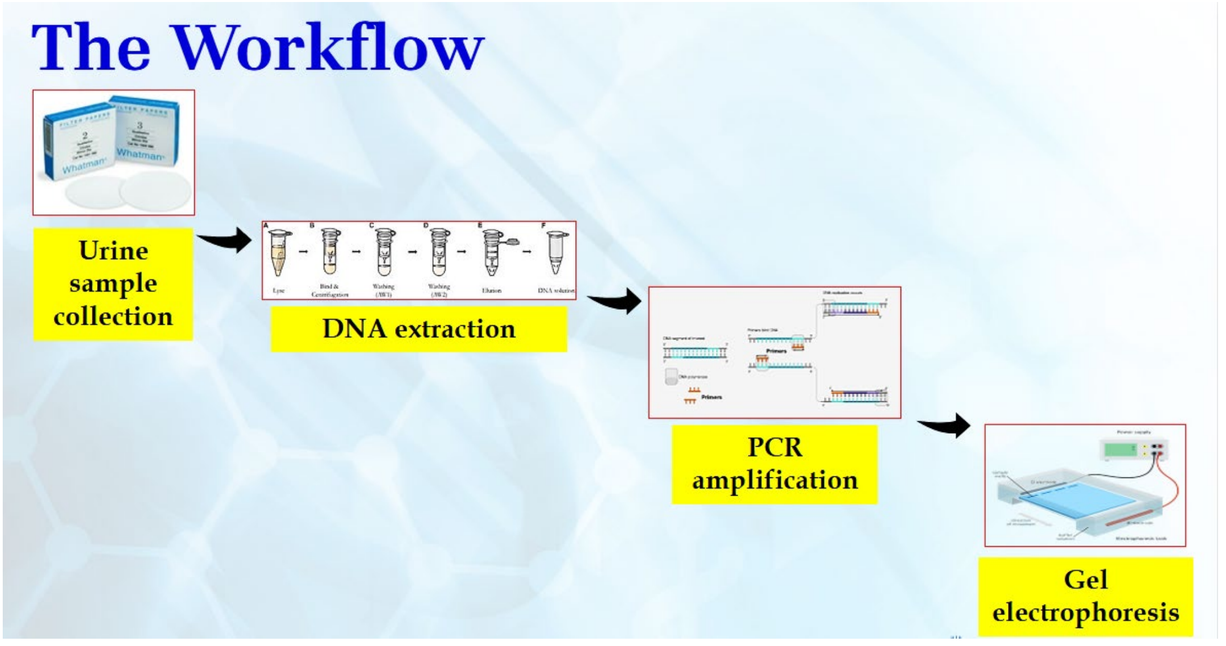


METHOD

- 7 urine samples were collected from Marquette University volunteers.
- Genomic hookworm DNA (gDNA) for *N. americanus* and *A. duodenale* was diluted into 9 different concentrations to represent 9 different levels of hookworm infection, which helps to ensure sensitivity.
- 4 different concentrations of primers (50 nM, 80 nM, 100 nM, and 250 nM) were tested for both repeats fragment DNA and *ITS-2* gene DNA for both species.
- These primer concentrations were added to a PCR mix with gDNAs for both species, control, and *Schistosoma* spp. gDNA for negative controls.
- The urine samples were then spiked with the 9 different concentrations of hookworm gDNA.
- The hookworm DNA was extracted, and the yielded DNA concentration was measured using a Nanodrop.
- These extracted hookworm DNAs of nine different concentrations from both species, yielding a total of 18 samples, will be run on PCR using the optimal primer concentration for both the repeat DNA fragments and the ITS-2 gene.
- These will all be run in duplicate, yielding a total of 72 sample tests.
- *Schistosoma* gDNA, DNA-RNA free water, a no-template control, and the opposite hookworm species gDNAs will be used as negative controls to ensure specificity, as well as the same species gDNA for a positive control.
- A true positive result would read as positive/positive, a true negative as negative/negative, while positive/negative results in a rerun.



WORKFLOW



- DNA extraction is done using Whatman #3 filter paper that is then hole punched and extracted using a QIAMP Blood Mini Kit.
- All electrophoresis gels will be run in duplicate to ensure the specificity of our assay.

RESULTS & DISCUSSION

- The primer concentration that yielded the prominent bands for repeat and *ITS-2* DNA across both species was 250 nM.
- *Necator* sample DNA ranged from 3 ng/ul to 26 ng/ul post dilution and 3.3 ng/ul-3.8 ng/ul post extraction.
- *Ancylostoma* sample DNA ranged from 3.4 ng/ul-128 ng/ul post dilution and 3.5 ng/ul-3.7ng/ul post extraction.
- Positive controls have shown amplification during various runs, with *Necator* showing the best amplification with the repeat primer at 57^{0}C.

CONCLUSION

- PCR assays for hookworm are much more sensitive and specific than microscopic methods like *KK and* can test a larger number of samples at the same time.
- Detection of hookworm from urine samples using PCR will greatly improve the accuracy and speed of species-specific detection, which will facilitate hookworm treatment worldwide

FUTURE WORK / REFERENCES

For our future work, we plan to run the PCR reactions for the hookworm species using the 250 nM primer concentrations for the 4 different sets of primers.

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