Histomonas ELISA

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Abstract:

Histomonas meleagridis is a protozoan parasite of avians and is the causative agent in Histomonosis, commonly known as Blackhead Disease. Current methods for diagnosing the presence of *H. meleagridis* are limited to parasite culture or Polymerase Chain Reaction (PCR) to amplify target DNA. This project aims to develop an enzyme-linked immunosorbent assay (ELISA) for faster and more sensitive diagnosis of Histomonas infections. Cultures of H. meleagridis parasites were purified, and surface antigens were extracted using a spectrum of chemical solutions. The various antigen solutions were subjected to an ELISA, with serum from birds immunized for *H. meleagridis* as positive controls. The ELISA was measured for absorbency differences between positive and negative control serum samples. Preliminary results indicate Propanol as a promising treatment; however conclusive data necessitates additional ELISA analysis. Histomonosis threatens the health of both wild and domestic bird populations. Control of this disease relies on quality management and diagnostic techniques. This ELISA will aid scientist, managers, and veterinarians in the study and eventual control of this disease, and also allow researchers the opportunity to understand the impact of Histomonas outbreaks and elucidate the attack rate of the parasite.

Literature Review:

Disease:

Histomonosis is a severe disease of turkeys and other gallinaceous birds caused by the protozoan parasite *Histomonas meleagridis* (fig. 1). These parasites are found in the ceca of infective birds, causing necrosis and inflammation (fig. 2). The parasites eventually leave the ceca and are transport via the circulatory system to the liver, causing characteristic circular liver lesions (fig. 2).

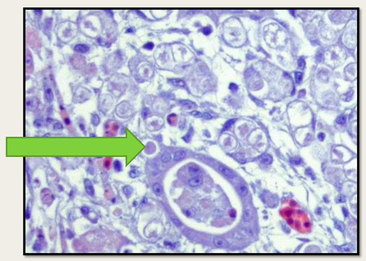


Figure 1. *H. meleagridis in a tissue sample.*

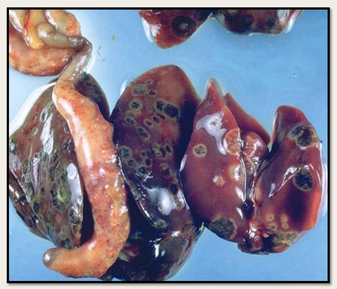


Figure 2. ceca inflammation and circular liver lesions characteristic of a *H. meleagridis* Infection.

Current diagnostics:

The literature shows that current diagnostic techniques for Histomonosis include culturing, Polymerase Chain Reaction (PCR) amplification of target DNA sequences, and culturing parasites for later identification using light microscopy.

Limitations:

- cannot be done in the field
- can take some time for results (up to 72 hours for culturing)
- must be done by an experienced technician.

Adding to the difficulty, the parasite easily degrades outside the host, so time to transport to the lab is a serious consideration.

Methodology:

To address this lack of proper diagnostic techniques, we are attempting to create an enzyme-linked immunosorbent assay (ELISA) for the presence of the parasite.

- First, we extract surface antigens from purified cultures of parasites using a polarity spectrum of chemical treatments (This was done with a similar parasite, *Trichomonas gallinae*) and subject them to a series of ELISAs.
    - Methanol, Ethanol, 2-Propanol, Acetonitrile, chloroform, diethyl ether, and hexane were used as the treatments.
    - *H. meleagridis* parasites were grown for 48 hours in Dwyer's media.
- Antigens were tested in the ELISA by blood serum samples of turkeys immunized for *Histomonas* (positive samples), or blood serum samples from turkeys not immunized against the parasite (negative controls).
- Results of the ELISAs were quantified by absorbency measurements.
    - The higher the absorbency the greater the positive response of the serum antibodies to the extracted antigen.
- We used a series of ELISAs to modify conditions of the ELISA to product a response that was both **sensitive** to the presence of the antibodies and **specific** to just the *Histomonas* antibodies.

Results:

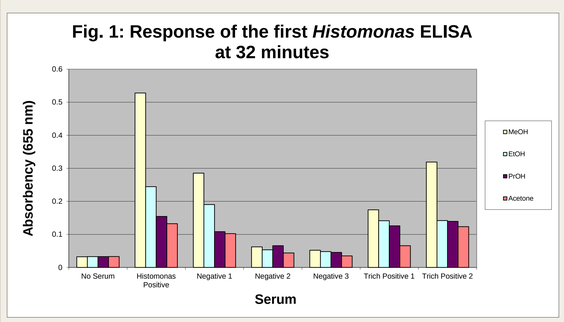


Figure 8 shows the response of the first *Histomonas* ELISA.

Methanol was the only tested treatment that passed the 0.4 absorbency threshold.

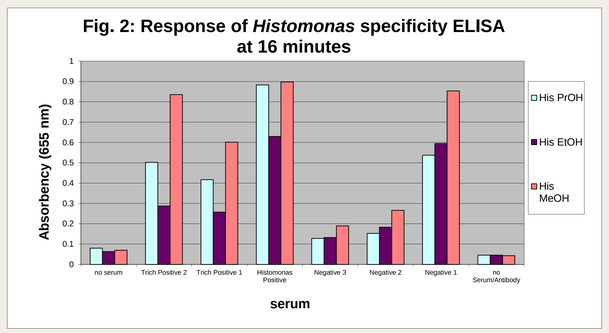


Figure 2 displays the response of the *Histomonas* specificity ELISA. A suitable antigen will show a high absorbency response for it's corresponding serum sample (Histomonas Positive), but low responses for negative controls and the other parasite serum sample (Trich Positive 1 and 2).

The ethanol treatment showed the best specificity under these criteria.

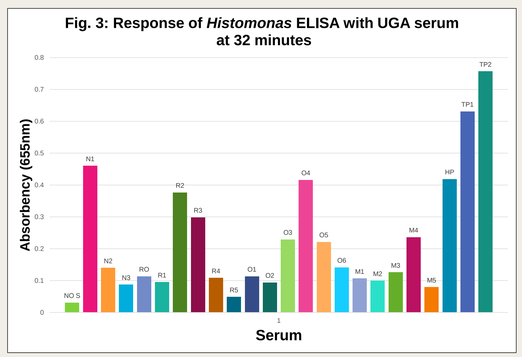


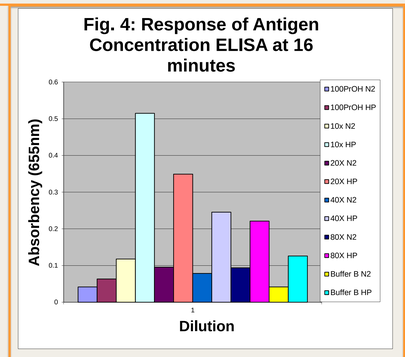
Figure 3 shows the response of the ELISA with additional blood serum samples provided by the University of Georgia. Samples N-R and TP 1 and 2 should be negative while samples O-HP should be positive.

Several of the negative UGA samples showed a stronger positive response than some of the positive UGA samples. Results suggest the ELISA needed more modification to accommodate the new samples.

Future work and Implications:

While current tests suggest alcohols as the preferred antigen treatment, we hope to continue with testing conditions for our ELISA before making any conclusive statements. Ultimately, we anticipate developing an in-field test using the antigen described in this report and ACEK-enhanced capacitive sensing methods developed by project contributors [1,2].

We are concerned about developing an ELISA for this disease, because the literature shows us **there is an increase in outbreaks of Histomonosis. Since all drugs previously used to treat the disease have recently been banned by the FDA, control of this disease relies on quality management which relies on quality diagnostics.** This is especially apparent in the field of wildlife research, where current diagnostic techniques are limited by time and funding. We hope this ELISA will address some of the limitations of current diagnostic techniques available as well as allow opportunities into researching the impacts of these outbreaks. The ELISA will be able to distinguish whether birds are recovering after these outbreaks, or if infected birds are simply dying of the disease. **This will give researchers a better understanding of the true impact of the disease on wild populations, which is a great challenge in the current literature.**



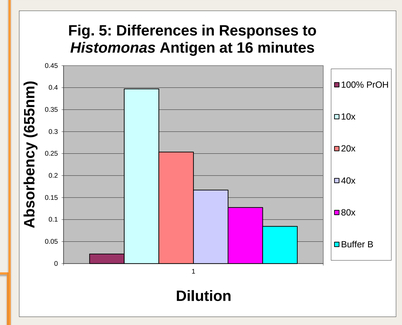


Figure 4 shows the response of various dilutions of the antigen solution. Figure 5 shows the difference in response of the antigen dilutions as compared to the negative control.

The 10X dilution showed the best response AND difference between positive and negative responses.

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References:

1. Liu, X., Cheng, C., Wu, J., Eda, S., and Guo, Y. (2017) A low cost and palm-size analyzer for rapid and sensitive protein detection by AC electrokinetics capacitive sensing, Biosens Bioelectron 90, 83-90.
2. Cheng, C., Wang, S., Wu, J., Yu, Y., Li, R., Eda, S., Chen, J., Feng, G., Lawrie, B., and Hu, A. (2016) Bisphenol A Sensors on Polyimide Fabricated by Laser Direct Writing for Onsite River Water Monitoring at Attomolar Concentration, ACS Appl Mater Interfaces 8, 17784-17792.

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