Histomonas ELISA
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Abstract:
Histomonas meleagridis is a protozoan parasite of avians and is the causative agent in Histomonosiosis, 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 detection of Histomonosiosis 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 scientists, managers, and veterinarians in the study and eventual control of this disease, and also allow researchers the opportunity to understand the impact of Histomonosiosis outbreaks and elucidate the attack rate of the parasite.

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 chemicals. This was done with a similar parasite, Trichomonas gallinarum, 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 produce a response that was both sensitive to the presence of the antibodies and specific to just the Histomonas antibodies.

Results:

Histomonosiosis 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 infested 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).

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.

Figure 1: Response of the first Histomonas ELISA at 32 minutes

Figure 2 displays the response of the Histomonas specificity ELISA. A suitable antigen will show a high absorbency response for its 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.

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 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-HF 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.

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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Figure 1: Response of the first Histomonas ELISA at 32 minutes

Figure 2: Response of Histomonas specificity ELISA at 16 minutes

Figure 3: Response of Histomonas ELISA with UGA serum at 32 minutes

Figure 4: Response of Antigen Concentration ELISA at 16 minutes

Figure 5: Differneces in Responses to Histomonas Antigen at 15 minutes

References: