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2012 Library Undergraduate Research Award

**Determination of Virulence Factors Associated with *Histomonas meleagridis* in Blackhead Disease in Poultry**

Three years ago, I joined the University of Georgia family with a meticulously designed plan. In four years, I would graduate from Franklin College of Arts and Sciences with a Biology degree. This degree would prepare me for my anticipated medical school, which would fulfill my aspirations of practicing human medicine as an orthopedic surgeon. Nowhere in this recipe for success did I intend to incorporate non-human subjects, especially not chickens. However, before I could blink, I had changed my major to Biological Sciences, which falls under the Poultry Science Department of the College of Agriculture and Environmental Sciences. My reasons were numerous, calculated, and logical; however, poultry research did not make the list. Research conducted involving or concerning poultry rarely makes the top of anyone's list of interest, including most research journals. This limitation required for me to call upon my inner Sherlock Holmes skills to sift through numerous inapplicable journals and publications. This need is where I found the UGA Libraries' resources to be most useful. Through meticulous filtering and the use of numerous available research tools, I was able to get a basis for my research, and prepare my publication on an epidemical disease that plagues chickens and turkeys.

My research focuses on a certain protozoan called *Histomonas meleagridis*. This microorganism is the lead cause for histomoniasis, or Blackhead Disease, which is extremely lethal to galliformes (poultry) by causing liver failure. This disease has

existed without a cure for over a century. To this day, little is known on how to treat Blackhead Disease. Dr. Robert Beckstead of the Poultry Science Department presented me with this research project as a continuation of a previous project dealing with *H. meleagridis*, which served as my foundation. My research is designed to characterize what virulence factors are in *Histomonas meleagridis*. In essence, I am attempting to find out what gives this disease its power. This predecessor project, conducted by Dr. Beckstead and Elizabeth Lynn, and helped design the vector I will use to test each virulence factor. I had a general idea of my method of action, but there were still many questions that needed answering before I could start running experiments. Using the UGA Libraries, I sought to find information on three particular areas: 1) what previous attempts and methods have been used to cure Blackhead Disease, 2) what are virulence factors and what virulence factors are common in diseases, and 3) what are ways used to fight a disease's virulence factors.

Before beginning any laboratory experiments, I analyzed the data presented by the previous publication, and used it as my starting point for further exploration of publications with similar subjects. I scanned through the bibliography to find articles that would be applicable to my particular project, and read through them to familiarize myself with *H. meleagridis*. These articles included studies about the molecular characterization of the protozoan, culturing the protozoan, and even an article written in 1922 inquiring into the nature of the protozoan. Knowing the history, characteristics, and life cycle of *H. meleagridis* aided me in laying the framework and foundation for beginning my research.

I began my quest for the answers of these three questions using a personal favorite database: Web of Science. This database is useful because it can be used as a springboard into other, more focused journals and articles. Besides allowing you to look at related articles or related reference work, Web of Science allows you to see where an article has been cited in later works. For a scientist, this tool is helpful because one needs to know the latest research in order to aid in one's research or to prevent plagiarism. Through Web of Science, I was able to find a myriad of articles that answered my first inquiry on previous methods of Blackhead Disease treatment by giving me the most up-to-date articles dealing with the disease. None of the previous attempts to discover a treatment had considered the use of studying the disease's virulence factors.

To find answers to my second question pertaining to what are virulence factors, I used Biological Sciences ProQuest. This database contained more simplified publications that were perfect in giving me a basic understanding of virulence factors, and how they influence organisms. Other databases I visited did not provide me concise answers because they were too focused on specific branches of science like agriculture or genetics. Once I broadened my scope, I was able to find adequate sources, and even able to compile a list of common virulence factors that I would insert into my vector to test for pathogenicity.

Several databases and publications helped give options to my third area of interest. Such databases included CAB, PubMed, and BIOSIS. Seeking for ways to inhibit or overcome virulence factors presented in *H. meleagridis*, I found previous studies, which used a wide host of techniques. One experiment used a technique

called quorum sensing to regulate virulence factors. Other experiments aided in my understanding of making vaccines or other medical treatments that would regulate the virulence factors of the disease.

Throughout my research I have learned several strategies in which to find the answers or information for which I am looking. The most efficient way to find the article that best fit one's research is to find the right database. Though there are a myriad of useful databases available via the UGA Library system, finding the specific one that applies to the research topic will give the most functional results. Upon finding the best-suited database, scanning the abstracts gives the assessment of a publication's usefulness.

Upon getting the foundational information on *Histomonas meleagridis*, Blackhead Disease, and the affect of virulence factors, I am able to begin effectively performing experiments and running tests. Although this disease that causes high mortality in poultry currently has no treatment, it is predicted that this study will provide insights that will lead to the cure.

Matthew Smith  
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CURO 2012 Symposium Abstract  
**Abstract**

Determination of Virulence Factors Associated with *Histomonas meleagridis* in  
Blackhead Disease in Poultry

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*Histomonas meleagridis* is the causative agent of Blackhead Disease in gallinaceous birds. It causes cecal inflammation and can spread to the liver leading to liver failure and death. This disease is known to have 80-100% mortality in turkey flocks. In a previous study, *H. meleagridis* was shown to lose virulence upon passage in culture suggesting a variation in pathogenicity that is also observed in the field. Our lab has identified potential virulence factors whose expression is lost upon passaging in laboratory cultures. To test the ability of these genes to cause disease, we are designing experiments to express putative virulence factors in a non-virulent strain of *H. meleagridis* via *transgenesis*. We are currently designing a transformation protocol specifically for *H. meleagridis*. My research project has been to design, generate and test this expression system. To do this, we cloned the *H. meleagridis* beta-tubulin promoter upstream of the neomycin resistant gene. A polyadenylation signal sequence was also cloned downstream of the neomycin gene to promote stable mRNA expression. Currently we are testing several electroporation conditions to assess the ability of this construct to confer resistance to G418. Upon successful transformation of *H. meleagridis* we will generate new vectors that contain sequences encoding virulence factors in place of the neomycin resistance gene and test their affect the pathogenicity of *Histomonas meleagridis* in turkeys. Virulence genes identified in these studies will be targets for future drug development and vaccine production.

## Bibliography in Progress

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Old techniques to fight H. meleagridis

1) detect antibodies against H. meleagridis

<http://www.sciencedirect.com.proxy-remote.galib.uga.edu/science/article/pii/S0304401710001858>

2) use of paromomycin

<http://www.sciencedirect.com.proxy-remote.galib.uga.edu/science/article/pii/S0304401709004063>

3) passaging in vitro to reduce pathogenicity

<http://www.sciencedirect.com.proxy-remote.galib.uga.edu/science/article/pii/S0264410X08006816>

4) use of drug w/ antiprotozoal activity

<http://www.sciencedirect.com.proxy-remote.galib.uga.edu/science/article/pii/S0304401704000998>