Expanding Horizons Proposal 2022

DVM Class of 2024

Project Title: Investigation of microcystins in free-ranging poultry around Lake Victoria, Kenya and their potential health implications in the context of Newcastle Disease Virus

Project Partners, Location, and Period

This project is proposed for a period of 8-weeks between June and July 2022, to be based in Kisumu, Kenya, and the surrounding villages, all along the shores of Lake Victoria. This Expanding Horizons project will be a collaboration between Cornell University College of Veterinary Medicine and the Kenya Marine and Fisheries Institute (KMFRI). My faculty mentor at Cornell University is Dr. Kathryn Fiorella, who has long-standing relations with research partners at KMFRI via her research initiatives focused on health and socioeconomic impacts of harmful algal blooms in Lake Victoria. KMFRI is a national research institute leading investigations into fisheries, aquaculture, marine research, as well as environmental and ecological dimensions. I am being welcomed by their Director of Freshwaters System Research, Dr. Christopher Aura, who will facilitate connections with poultry-owning households within their research networks and provide technical support during my stay. Remote mentorship will also be provided by Dr. Amber Roegner, a veterinary toxicologist who conducts research with the Fiorella Research Group and KMFRI.

Purpose

Since the conception of the term "Planetary Health," there has been a heightened awareness of the impact anthropogenic activities have on the climate and environment, and what these perturbations mean for human and animal populations. It is simply a matter of picking a region to then discover what challenges plague the systems in that area. From an aquatic lens, there is a growing issue: harmful algal blooms (HABs), especially those producing cyanobacteria. HABs are found world-wide and are increasing in prevalence due to climate change and anthropogenic nutrient pollution [1-3]. In the Kisumu Bay region of Lake Victoria where I will be based, human waste pollution is the major nutrient input that fuels cyanobacterial proliferation [2-4]. These algae, in particular, are of concern due to a class of toxin that they produce: microcystins (MCs). An extensive body of research reflects the scope of damage that MCs exert; MCs target the liver, brain, kidney, lung, heart, and reproductive system [5-7]. Acute exposure to MCs via HABs has led to fatal outcomes for humans and animals [5]. On the other hand, health effects stemming from chronic exposure to MCs are not well understood aside from evidence pointing to various types of cellular damage [5-7]. One possible route of exposure to MCs is via contaminated drinking water [3,5,8]. Another exposure risk factor is ingestion of fish [2,9]. Lake Victoria boasts an important freshwater fishery, and the fishing communities that line the shore rely on this supply of essential animalsource food, which contributes omega-3 fatty acids and micronutrients to their diets [10]. One dimension of the Fiorella Research Group's work is to assess MC accumulation in fish tissues across species to better understand what health risks exist from eating fish. It is of utmost importance to understand these risks because individuals might be paradoxically harming their health in pursuit of essential nutrition. The fear is, though, that the scope of damage that HABs and MCs pose is minimally known and extends beyond the fish-human axis.

Within these fishing communities, households own free-ranging chickens. Observations from the field suggest that poultry may be exposed to MCs from drinking lake water or from being fed dagaa (*Rastrineobola argentea*), a sardine-like fish that is commonly eaten by people but believed to accumulate MCs in its hepatopancreas [2]. Poultry in these fishing communities are not a staple source of animal-source food, but we cannot neglect their role in MC dynamics in the food web as well as the impacts MCs have on their health. The literature focused on avian species in relation to HABs and MCs is scant, with

many of the publications reporting bird mortality events (*e.g.*, flamingoes, pelicans) due to MC exposure [11-14]. The results of one laboratory study in Japanese quail suggest that exposure to high-dose cyanobacterial mass, while producing more prominent lesions than what was seen with levels equated to environmental levels of MCs, would not have been sufficient to kill the birds; rather, they proposed that avian die-off events linked to HABs work in a manner where the effects of MCs and other stressors combine to yield mortality [15]. At the very least, there is a link between MC exposure and physiological stress levels (ascertained from heterophil:lymphocyte ratios), which has the potential to make a bird susceptible to another insult, like a pathogen [16].

The final dimension to understand that is underlying this proposal is what threats poultry face in Kenya. In a study based in western Kenya, the average flock size per household was ten chickens, a number largely constrained by mortality [17]. Newcastle Disease Virus (NDV) is a leading cause of chicken mortality globally, and it is believed to underlie in large part the high mortality rates that household poultry flocks face. The majority of Kenya's poultry scene consists of free-ranging indigenous breeds or crosses with exotic breeds, while a smaller fraction represents commercial broilers and layers kept in caged, commercial operations. Free-ranging poultry in village settings are usually not vaccinated against NDV, and as such, NDV is endemic and widespread in Kenya [18]. In the context of the fishing communities whose free-ranging poultry are potentially being exposed to MCs, there is fear that NDV is more devastating to their poultry. Our project in collaboration with KMFRI is posed to begin a rudimentary investigation that brings together the pillars of the environment change, animal health, and their effects on poultry-owning households.

Project Objective/Hypothesis

The overarching goal of this research project is to broaden our understanding of MC impacts on food systems within the context of free-ranging poultry. A primary objective is to assess whether free-ranging poultry inhabiting lake-side fishing communities are exposed to MCs. The hypothesis is that poultry drink lake water, which during periods of high cyanobacterial algal blooms is an exposure risk. The other proposed source of exposure to MCs is via consumption of dagaa or other fish scraps via supplemental feeding efforts. Making that correlation will establish dagaa as a multi-species MC exposure risk factor. The second crucial objective is to determine the seroprevalence of Newcastle Disease Virus in the villages-both lakeside and inland-where sampling occurs. Before trying to attempt to make links between MC exposure and NDV disease status, we need to understand the epidemiology of NDV in these household flocks. It is projected that poultry exposed to MCs will be under higher physiological stress, reflected via an increased heterophil to lymphocyte ratio. Overall, poultry exposed to MCs should experience higher levels of physiological stress and be more likely to succumb to NDV infection. The results of this study have the possibility to inform future studies aimed at better understanding the role of MCs in poultry. A concurrent dimension to the project includes household interviews geared towards learning about poultry husbandry parameters and concerns about poultry health. By developing relationships with community members during the summer, I will be able to recommend minor changes to improve husbandry (e.g., constructing a prototype shelter) and educate individuals on NDV clinical signs and how to minimize losses within their flock.

Methods/Strategy

The project aims to assess whether MCs are of concern to free-ranging poultry, and if so, whether this can be detected as a heightened physiological stress level. Simultaneously, NDV status within village poultry populations will be investigated to explore correlations between the MC and NDV variables. The research is anticipated to be implemented in phases to ensure a sustainable approach to community-based work. Within the KMFRI research infrastructure and the long-standing relationships the Fiorella Research Group has with households in the Kisumu Bay region, I will be connected with poultry-owning households that are willing to be interviewed and have their chickens sampled at a later date. Building

trust and understanding their perspectives and concerns about their poultry's productivity and health will be crucial. To ensure that the research questions about MC exposure can be addressed appropriately, we will select villages directly along the lakeside, as well as villages more inland where free-ranging poultry are not in direct contact with the hypothesized MC exposure points (i.e., lake water, dagaa). Thus, lakeside villages will be the experimental group, with inland sites serving as a control group. A translator will assist me in the field to ensure effective communication and documentation of survey data. The survey will collect information ranging from flock size, supplemental feeding (especially regarding dagaa), breed, to health concerns. Translated infographics about NDV clinical signs will be used to guide health questions and raise awareness of this important pathogen. Direct observations will be made at each site to learn patterns of poultry movement and assess whether poultry are interacting with MC risk factors. For the sampling phase of the project, the goal will be to sample as many chickens as possible given the stock of sampling supplies. Households that coop their chickens up at night will be asked to keep them enclosed in the morning to facilitate sampling. Birds will be photographed for identification purposes (sex, breed, juvenile versus adult), assessed for NDV signs, and lastly a blood sample will be obtained for serum in a red top tube and two blood smears will be prepared. Serum will be subsequently tested via an NDV-antibody detecting ELISA and a MC detecting ELISA; all samples will be run in duplicate. We are anticipating that most chickens will not be cooped up for easy sampling, in which case we will recruit and incentivize help from children. KMFRI's Kisumu location has laboratory space and equipment (microscope, spectrophotometer) needed to perform ELISAs and read blood smears. I plan to run ELISAs at KMFRI, but we are developing a back-up plan by storing leftover serum samples in the event that tests fail at KMFRI. There are numerous variables that could go wrong with an ELISA even outside of the context of transporting a kit overseas and working in a limited capacity laboratory. If samples need to be exported to the U.S. for analysis domestically, we will pursue those logistics as they arise. The data will be recorded and analyzed for statistical significance between locations deemed "lakeside" versus "inland." These efforts will be repeated for each village where sampling occurs. Data that are obtained will inform subsequent discussions with poultry-owning households about poultry management and disease risks. The solutions may be limited in nature, but our goal is to address the concerns stakeholders share with us prior to sampling so that they may implement minor changes in their procedures to ameliorate poultry health and production parameters, which subsequently impact their own livelihoods and nutrition.

Approvals/Permits Needed

Given that the primary plan is for all analyses to be carried out in Kenya, no samples will need to be transported back to Cornell. As such, no permits will be required for this project. If plans shift out of necessity to conduct laboratory analyses back in the United States, permits will be required to transport serum samples. I will be working with Dr. Fiorella to procure the necessary permits.

Feasibility, Expected Outcomes, and Difficulties

International projects inherently have numerous challenges associated with them, which is something I have had exposure to via field work in Indonesia, China, and the Dominican Republic. With that background, we sought to design a project that has the potential to reveal a significant and novel finding without a complicated protocol to achieve the research objectives. With that said, I understand that the research itinerary represents an ambitious endeavor for an eight-week period. Since the project has multiple layers of investigation, there is a built-in safety mechanism to ensure some meaningful data can be collected even if some dimension of the project fails or cannot be completed as proposed. My mentors both in the U.S. and in Kenya have long-standing relations with the villages where I hope to conduct sampling, which will facilitate trust and increase the likelihood of interest and cooperation with the project. I have proficient avian venipuncture skills, and previously conducted a project during undergraduate where I caught common eider ducks and bled them for serum samples. I would utilize the infrastructure at Cornell Vet to hone my blood smear skills, and ensure I can adequately fix, stain, and

read the slides. My current project led by Drs. Radcliffe and Dhondt has afforded me the opportunity to develop growing competency with avian white blood cell identification. I would also seek practice in running an ELISA to ensure I am familiar with the procedure before running them at KMFRI. One outstanding risk is a kit becoming compromised in transit to Kenya, or inconsistent temperatures during the protocol that lead to inconsistent and unreliable data. As mentioned above, the workaround solution to these difficulties will be exporting stored serum for analysis in the U.S. Additional difficulties that are expected to arise include catching poultry. Should they prove too difficult to catch even with recruited help, we will be prepared to bait chickens with supplemental grain feeding and opportunistically trap them. I also acknowledge that the language barrier may pose a challenge. I currently have no proficiency with Dholuo, though I am committed to self-study to build some conservational capacity, as I have seen first-hand how much more meaningful community-engaged international efforts proceed with attempts to bridge the language barrier. A translator familiar with Dholuo and English will be utilized for surveys to ensure effective communication and data collection. English is recognized as an official language in Kenya, and numerous individuals at KMFRI are fluent in English; as such, I believe the project will not be hindered by language. We designed this study acknowledging limited resources and related constraints, so we believe that via this collaboration, we will be able to achieve our objectives of assessing whether poultry factor into the multi-modal web of effects MCs play on humans and animals alike, and what this means for the prevalence of an already devastating pathogen.

Professional Impact and Development

I aspire to become a veterinary researcher within the domain of planetary health, where I can draw upon my deep-rooted interests in avian species, disease dynamics, and conservation, and engage them in addressing human health concerns at the human/wildlife interface. My interests have shifted since being admitted to Cornell Vet, and this is largely due to my admiration of the interconnectedness of One Health and planetary health. Although I theoretically knew the wide scope of impact a veterinarian could have via their career, it took cultivation in Cornell's rich research environment to connect with individuals like Dr. Fiorella, who make meaningful impact via their research initiatives. My veterinary training is providing me with skills ranging from avian handling and restraint to disease transmission across species. Conservation medicine is an umbrella term many students like myself enter veterinary school hoping to pursue; however, conservation medicine is more than just technical skills and the veterinary science behind treatments. One cannot consider animals outside of the context of their habitats, the perturbations humans cause to their habitats, the underlying motives behind those human acts (e.g., driven by survival), and the social institutions within which all these spheres intersect. The wide array of links across domains excites me and inspires me to pursue additional training via an M.P.H. and a Ph.D. in Global Health following veterinary school. I am compelled that partaking in this Expanding Horizons opportunity would afford me an opportunity to put my newly evolved interests to the test in the field. The ambitious nature of this project reflects the desire to utilize this summer as a pilot study for potential Ph.D. work, as I will get to know the people, the samples, the landscape, and begin to navigate difficulties. I foresee community-based, international field work as a crucial pillar in my future career, and this project will allow me to continue developing cultural and scientific fluency. It is inevitable that an experience of this nature, similar to a prior Engaged Cornell experience in Indonesia, will have a formidable impact on my career trajectory. I will gain exposure to poultry medicine, veterinary toxicology, and international public health, while also appreciating the corollary work at KMFRI focused on HABs, fisheries, and food security. I am eager to continue growing as a future veterinary researcher under the guidance of Drs. Fiorella, Roegner, and Aura. An opportunity of this nature this early in my training will be careerbuilding and will empower me to continue acting on my passions such that I can join the network of planetary health researchers, who have been such an inspiration to me.

References

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Budget

Travel	Cost
Round-trip flights (JFK - AMS - NBO - KIS)	\$1,241
Single Entry Travel VISA	\$51
COVID-19 PCR Screening Tests (x2)	\$100
Field Work Logistics	
Ground transport to field sites	\$500
Translator fees (Dholuo and English abilities)	\$300
Living Expenses	
Housing - Kisumu Terrace Apartment Rental via Airbnb (for 8 weeks)	\$781
Food - breakfast (\$5), lunch (\$5), dinner (\$10) per day	\$1,120
Personal items (e.g., insect repellant, first aid kit)	\$40
Research Materials	
BD 3 mL syringe with 25 gauge, 1" needle (100/box, x2 boxes)	\$39.98
3mL red top tubes (100/pack, x2)	\$77.98
Glass microscope slides (144/pack, x2)	\$23
Glass cover slips (69/pack, x4)	\$37.48
Microscope slide box (100-slide boxes, x3)	\$34.62
Methanol	\$7.60
Diff-Quik Stain Kit	\$119
IDEXX NDV-Ab ELISA Test Kit (5 plates, 96 wells/plate)	\$487.02
Abraxis Microcystins/Nodularins (ADDA), ELISA Test Kit (96-test/kit) (\$500/kit x 2)	\$1,000
Preventative Medicine	
Yellow Fever Vaccine	\$165
Cholera Vaccine	\$230
Doxycycline (Malaria prophylaxis)	\$35
Additional medications for safety measures (e.g., Loperamide, Ibuprofen)	\$20
TOTAL:	\$6,409.69

Budget Justification

This budget totaling to \$6,409.69 has been prepared via consultation with my mentors and partners involved with this project, in addition to discussion with a previous trainee of Dr. Fiorella's who planned an Expanding Horizons project in Kisumu, Kenya. Flight costs reflect the current state of prices for round trip travel from New York (JFK) to Kisumu (KIS) by way of connections in Amsterdam (AMS) and Nairobi (NBO) with an early June departure and return at the end of July. Housing costs were obtained from an apartment rental via Airbnb, which for the length of time requested, yields substantial discounts. Ground transportation using local means of transport will be essential to reach field sites and was

estimated via consultation with our partners. A translator will be needed for surveys, and we are hoping to utilize a KMFRI intern as a lower-cost option and coordinate with other researchers there for the summer to pool together funds for our translator needs. Research material costs were obtained from sources including Amazon, Grainger, Fischer Scientific, IDEXX, and other lab supply companies; I sought out affordable options that would still ensure the work can be carried out appropriately. The IDEXX ELISA kit listed comes with five plates, which is a larger number than required for anticipated sample sizes, but IDEXX sales representatives were unable to provide an option to obtain a smaller kit. The Abraxis ELISA kits are a substantial expense, but they lie at the core of the main research objective that could lead to a novel finding. COVID-19 testing requirements for entry into Kenya were considered, and I will be using a Cornell supplemental PCR test prior to departure, and will utilize a diagnostic lab in Nairobi before returning to the U.S. The CDC and Cornell Health were referenced for guidelines and costs pertaining to preventative medicine. In compliance with the recommendations, I would need to obtain the Yellow Fever and Cholera vaccines; my other vaccinations are up to date. We acknowledge that the grant is capped at \$5,500, including a \$500 maximum for research supplies, both of which have been exceeded. We kindly request that the committee consider this proposal for the maximum award of \$5,500, and we will seek additional funding to cover the remaining expenses.

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KISUMU CENTRE

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Date: 09/02/2022

REF: KSM/KMFRI/2022/Vol. IV

Dear Expanding Horizons Committee,

REF: SUPPORT LETTER FOR

I am writing this letter in support of proposal titled "Investigation of microcystins in free-ranging poultry around Lake Victoria, Kenya and their potential health implications in the context of Newcastle Disease Virus" to be submitted to the Expanding Horizons Program at the Cornell University College of Veterinary Medicine. We plan to warmly welcome during the summer of 2022.

At the Kenya Marine and Fisheries Research Institute (KMFRI) in Kisumu, the research we conduct is wide-ranging, from fisheries and aquaculture to environmental and ecological studies. will be extending the scope of our research to investigate links between the microcystins from harmful algal blooms known to be a health risk and seeing if free-ranging poultry are exposed to these toxins. He will be working with fishing communities that we have strong and long-lasting relationships with before proceeding with sampling. He plans to then see if there are any ties to poultry health. Given that KMFRI is committed to sustainable development of the blue economy, we are happy to support in providing sustainable recommendations for households keeping poultry.

As the Director in charge of Freshwater Systems Research and other researchers in my department at KMFRI, we plan to facilitate introductions between and poultry-owning households and support his research during his stay in Kisumu. This work has the potential to expand our understanding of the multi-dimensional risks microcystins play beyond fish, so we are eager to take part in this preliminary investigation.

We look forward to mentoring in this endeavor and hope that you will find his grant proposal worthy of receiving funding. We believe this will be a great opportunity for to develop an appreciation for global health research, and it will be a chance for us to welcome fresh perspectives on the scope of harmful algal blooms on human and animal health.

Thank you for your time and consideration of proposal.

Dr. Christopher M. Aura (PhD)

DIRECTOR FRESHWATER SYSTEMS RESEARCH