

Thesis

Avian Aspergillosis: Experiences in Raptor Rehabilitation

Submitted by

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Introduction

Raptor rehabilitation is a critical component of raptor conservation efforts. By helping sick and injured individuals recover, rehabilitation helps to support populations of raptors that are decreasing globally. Over the past summer, I was given the opportunity to intern with the rehabilitation department of the Rocky Mountain Raptor Program located in Fort Collins, Colorado. As someone whose career goal is to work in wildlife or conservation medicine, this internship was an invaluable experience. It not only expanded my knowledge of the care of wildlife species, but also allowed me to apply what I have learned in my two majors, zoology and biomedical science, in real life scenarios while also building upon the content from my classes. One topic in particular encapsulated what I enjoy most about zoology and biomedical science: aspergillosis.

Raptor Rehabilitation

The group of birds called raptors, also known as the birds of prey, represents only 5% of all known avian species.¹ This includes the Accipitriformes (hawks, eagles, old world vultures, kites, and osprey), Cariamiformes (seriemas), Cathartiformes (new world vultures), Falconiformes (falcons and caracaras), and Strigiformes (owls).¹ Globally, of the 557 identified raptor species, 18% (103) are considered threatened (critically endangered, endangered, or vulnerable), an additional 13% (70) are near threatened, and 52% (292) have declining populations including 38% of the 142 species identified as least concern.^{1,2} On the other end of the spectrum, only 9% (49) of raptor species have increasing populations.² While they only represent 5% of total avian species, raptors are overall more threatened than birds in general.² Among the raptors, the old world vultures have the highest risk of extinction, as 12 of the 16

known species are currently recognized as endangered or critically endangered.² A majority of threatened and declining species are located in South Asia, Southeast Asia, Sub Saharan Africa, and South America.²

The status of raptors specifically in the US and Canada is much more stable. The most threatened raptor in the US and Canada is the California condor (*Gymnogyps californianus*) which is recognized as critically endangered by the IUCN.¹ Other priority species according to the IUCN are the 'Io (*Buteo solitarius*), spotted owl (*Strix occidentalis*), and snowy owl (*Bubo scanndiacus*).¹ While the number of priority species appears low, the ICUN looks at global populations and thus does not consider the regional conservation status of some species.¹ For example, the American kestrel (*Falco sparverius*) is experiencing sharp population declines in the Northeast US, the golden eagle (*Aquila chrysaetos*) is a species of concern in the western US and Canada, and the short-eared owl (*Asio flammeus*) is almost entirely absent from its previous range in Pennsylvania.¹ The American kestrel and short-eared owl are experiencing the largest population losses among species of raptors in the US and Canada.¹ Despite declines in most populations, some raptor populations are increasing. Cooper's hawks (*Astur cooperii*), bald eagles (*Haliaeetus leucocephalus*), and osprey (*Pandion haliaetus*) all have increasing populations, however this is mostly associated with recovery from population declines due to the prior use of DDT.¹

Raptors are more sensitive to human made threats and extinction than other avian species.¹ This is due to their position higher up on trophic levels and slow life histories leading to smaller and slower growing populations.² In addition, due to their low population densities, it is

difficult to observe and monitor populations.² The largest threats to raptor populations are habitat destruction, intentional killing, both intentional and unintentional poisoning with lead and rodenticides, electrocution, and climate change.^{1,2} Declines in raptor populations due to these threats can have environmental consequences due to their roles in ecosystems as predators and scavengers.³ As predators, they exert top down regulation of prey, while as scavengers, they consume carcasses and organic waste helping to prevent the spread of disease and aiding in nutrient recycling.^{1,3} The loss of predators and scavengers from ecosystems has been associated with the loss of plant diversity, biomass, and productivity.³

Along with their roles in regulating ecosystems, raptors can provide valuable ecosystem services to humans. Firstly, they can help regulate zoonotic diseases by feeding on infected hosts as well as competing against hosts for resources.³ By feeding on carriers of zoonotic diseases such as rats, rabbits, and prairie dogs, raptors help to limit the prevalence of disease in host populations while also limiting the number of individuals in the diseased population, lowering the likelihood of interaction with humans.³ In addition, some raptor species such as vultures compete with hosts of zoonotic diseases for resources such as carrion.³ One study found that in India, a decline in vulture populations due to the use of diclofenac in agriculture was linked to an increase in stray dog populations, increasing the risk of dog bites and the spread of rabies.³ Furthermore, a decline in vulture populations opens up their ecological niche to other scavenger species that pose a greater threat of spreading zoonoses to humans such as rats.³ Due to their consumption of carrion, vultures are also important in carcass removal helping reduce disease risk in livestock herds.³ Raptors can also indirectly increase agricultural output by controlling populations of pests. For example, American barn owls (*Tyto alba*) in California have a diet that

consists almost entirely of common agricultural pests and thus play a large role in regulating their populations and reducing crop damage.³ Similarly, one study showed that the presence of New Zealand falcons (*Falco novaeseelandiae*) near wineries in New Zealand, increases winery output due to their predation of birds that consume the crops.³ The loss of raptors can disrupt the ecosystem services they provide, leading to a negative impact on human wellbeing.²

Because of their importance in ecosystem health and in providing ecosystem services, the conservation of raptors is an important field. Some raptors with critically endangered and endangered populations are involved in captive breeding programs such as the Mauritius kestrel (*Falco punctatus*), the European bearded vulture (*Gypaetus barbatus*), and the California condor.^{1,4} These programs are working to grow stable captive populations that can then be used to grow and supplement wild populations of these species. For other more stable populations, rehabilitation programs are important in aiding raptors and their populations on an individual level by taking in sick and injured birds that may otherwise die and allowing them to recover before being released.

Roles and Responsibilities

The Rocky Mountain Raptor Program is a raptor rehabilitation and education center that is located in Fort Collins, CO that provides year round volunteer opportunities as well as a seasonal summer internship. As part of my internship, I worked 20 hours a week and was involved in multiple aspects of patient care. Shifts would begin with the organization of patient records and the morning bird check during which it was my responsibility to check on each patient to make sure they were bright, alert, and responsive and had no new medical issues that

needed to be reported to staff. In addition, bird check was used to ensure that the enclosures were clean and safe. After morning tasks, I would move on to feeding the diurnal birds and cleaning enclosures. During this time, I would also get the opportunity to catch, restrain, and assess birds to help monitor their health and recovery. Later on in the internship I was allowed to administer medications orally and subcutaneously to some patients.

After all birds were cared for, I would begin taking care of the mouse and rat colonies used for live prey testing of the birds. This entailed refreshing their food and water, cleaning their enclosures, and weaning and sexing babies. Alternatively, I could begin working on food prep for the patient's meals for the next day. Finally, if there was time during the day, I got to perform necropsies on any birds that had passed away or needed to be euthanized. During the necropsies I was responsible for performing the dissection and the physical, external, and internal examination of the bird, as well as recording my findings in proper documentation. A day would end with a final bird check and feeding of the nocturnal birds.

In addition to all of my responsibilities during my normal shifts, I was required to take classes about various aspects of raptor rehabilitation. The first classes focused on raptor anatomy and physiology which are essential for understanding certain injuries and illnesses. Additionally, knowing the anatomy of raptors is useful when assessing birds or performing necropsies. The next class focused on diet and nutrition, which went over the different dietary requirements of each type of raptor that can be admitted to the rehab center. This was important for properly doing food prep and making sure that each patient was receiving the appropriate diet. The classes on raptor behavior, natural history, and identification provided information on all of the raptors

that have ranges in Colorado including their aggression and stress levels in captivity, migratory patterns, and differentiating species from one another. The final class detailed the most common diseases afflicting raptors in rehabilitation programs, including those caused by issues with management and those caused by infections. As a student studying both cardiopulmonary physiology and zoology, the one disease that piqued my interest was avian aspergillosis because of its status as a zoonotic respiratory infection.

Avian Aspergillosis

Aspergillosis is a noncontagious fungal infection caused by species in the genus *Aspergillus* that can impact a vast range of animals including mammals and birds.^{4,5} *Aspergillus spp.* are saprophytic molds that are globally distributed, ubiquitous in indoor and outdoor environments, and play an important role in nitrogen and carbon recycling.^{5,6,7,8,9,10} Of the over 260 species of *Aspergillus*, most infections are caused by *Aspergillus fumigatus* due to its small conidia, high thermotolerance and pH tolerance, and low nutritional requirements.^{5,6,8,9} Infection can also be caused by *A. flavus*, *A. niger*, *A. nidulans*, and several others.^{5,6,9,10,11} The most common site of infection is the respiratory system which occurs due to the inhalation of conidia, the asexual spores of the fungus.^{4,7,8,10} Raptors can be exposed to conidia while consuming infected prey or from conidia present in the soil and air.⁶

In mammals, aspergillosis occurs only in the severely ill or immunocompromised.¹¹ Most birds that develop aspergillosis are stressed or immunocompromised, but it is also possible for healthy birds to develop it if they inhale a massive amount of spores.^{6,9,10,11,12} Birds are more susceptible to aspergillosis than mammals due to their unique lung anatomy and

physiology.^{5,8,9,11} Firstly, birds do not have a diaphragm so they are not able to forcibly expel small particles such as conidia from their respiratory system.⁹ They also do not have an epiglottis, which in mammals helps to prevent matter from entering the airways.^{4,9} The air sacs of birds have minimal local immune cells and very poor vascularization, making the recruitment of immune cells to the area more difficult.^{8,9} The immune cells that are recruited include heterophils instead of the mammal's neutrophils, which may be less effective at fighting off fungal infection.⁸ The environment of the air sacs additionally promotes growth of both the hyphal and sporulating forms of the fungus whereas mammals only develop hyphal growth.⁹ This means that in birds, more conidia are being produced inside of the respiratory system that can then proceed to further infect the individual. Finally, the respiratory system of birds has very little ciliated respiratory epithelium leading to limited mucociliary function and reduced clearance of particles such as conidia from the lungs.^{8,9}

Avian aspergillosis, also known as brooder pneumonia, pseudotuberculosis, asper, and mycotic pneumonia, is one of the most common infectious diseases that impacts raptors.^{6,7,10,12} It can manifest in two different ways in the respiratory system of birds. The first is acute aspergillosis which occurs due to the inhalation of an overwhelming number of conidia.⁴ Acute infection can rapidly progress and is often fatal.^{7,10} The second is chronic aspergillosis which occurs due to low level exposure to conidia in immunocompromised individuals.⁴ This immunosuppression can happen as a result of stress, poor ventilation of an enclosure, neonatal or geriatric status, corticosteroids, respiratory irritants, and lead poisoning.^{4,10} Both the acute and chronic forms of aspergillosis also have the potential to disseminate from the respiratory system to other organs which is often fatal.⁴ Because of its high mortality rates, avian aspergillosis is the

most common cause of death in captive and recently captured raptors.⁶ It has also been identified as the most important cause of mortality in the European Bearded Vulture Project, a captive breeding program for the critically endangered European bearded vulture.⁴

There are numerous risk factors associated with the development of avian aspergillosis. Though all birds are susceptible to aspergillosis, certain groups have a higher susceptibility including parrots, mynas, waterfowl, marine birds, captive penguins, and captive or recently captured raptors.^{5,7,10,13} In raptors, some of the species with the highest risk of infection are gyrfalcons (*Falco rusticolus*) and gyrfalcon hybrids, merlins (*Falco columbarius*), goshawks (*Astur gentillus and Astur atricapillus*), bald eagles, golden eagles, black sparrow hawks (*Astur melanoleucus*), grey falcons (*Falco hypoleucos*), immature red-tailed hawks (*Buteo jamaicensis*), and rough-legged hawks (*Buteo lagopus*).^{5,6} Aspergillosis in owls is rare but can be a concern for northern species such as the snowy owl.^{5,6,12}

Avian aspergillosis is more commonly diagnosed in captive or recently captured raptors than in free ranging raptors, although outbreaks can occur if several individuals are exposed to large numbers of conidia at the same time.^{5,6,7,9,11} These outbreaks are associated with environmental conditions that promote fungal growth and or spread, and have been reported in some raptor populations.^{9,11} Most outbreaks occur in the fall or early winter although individual cases can occur at any time during the year.⁵ Wet weather followed by dry conditions or drought can cause an increase in conidia in the environment because *Aspergillus spp.* reproduce during wet periods and release conidia when it becomes dry.^{5,7} Increases in airborne dust associated with dry conditions are also known to interfere with respiratory clearance of conidia, further

increasing susceptibility to infection.⁷ Individual infections are more common in raptors that are stressed, malnourished, or have a concurrent disease.^{6,7} While aspergillosis isn't commonly reported among wild raptors, data is generally insufficient meaning it is likely more common than is currently recognized.^{5,7}

Outbreaks in wild populations can be harmful especially if they occur in a species with a small population as it can result in inviability or extinction of the population.¹¹ For example, in 2019, there was an outbreak of aspergillosis in a population of Kākāpō (*Strigops habroptilus*), a critically endangered species of parrot that has a population of only 200 individuals.¹¹ This outbreak affected 21 birds representing 10% of the total population and resulted in the death of a total of nine breeding females, chicks, and juveniles.¹¹ While in most bird populations, this loss of individuals may be inconsequential, in a critically endangered population, losing even one bird can severely hinder conservation efforts. Thus, aspergillosis outbreaks should be a major concern for critically endangered species of raptor such as the California condor and the European bearded vulture.

Young birds are particularly susceptible to aspergillosis.^{5,10} Young red-tailed hawks between the ages of two and four months have the highest risk among young raptors.⁵ Outbreaks of brooder pneumonia occur during the spring.⁷ Brooder pneumonia usually occurs when an egg breaks before hatching, providing an ideal environment for fungal growth and subsequent infection of newly hatched chicks.^{5,7} Brooder pneumonia is of particular concern for captive breeding of endangered species as it can lead to detrimental losses of chicks.⁷

Noninfectious conditions such as trauma, gunshots, oiling, emaciation, exhaustion from migration, and poisoning from pesticides or heavy metal can increase risk of aspergillosis.^{5,12} This is especially important in populations such as the California condor that are critically endangered and are particularly threatened by lead poisoning as development of aspergillosis can lead to population losses.^{1,10} In captive raptors, overcrowding, poor sanitation, poor ventilation, inadequate diet, and exposure to respiratory irritants may also contribute to increased susceptibility.^{5,10} Because of this, it is important to make sure birds are being fed proper diets and that enclosures are cleaned regularly, birds are housed individually or in large enclosures, and organic debris such as sticks and leaves are removed.

The signs and symptoms of avian aspergillosis are non-specific, with similar symptoms appearing for other diseases and conditions, namely those that also impact the respiratory system.^{5,8,9,10} All of the common symptoms, excluding difficulty breathing also resemble those seen with lead poisoning.^{5,7} This can potentially lead to misdiagnosis if respiratory symptoms are subtle or missed. In addition, symptoms usually do not present until the disease is advanced.^{4,8} The combination of these factors makes it more difficult to catch and diagnose the disease early, leading to a delay in treatment which can have negative impacts on patient outcomes. This is due to the rapid progression of the disease, with acute pulmonary aspergillosis becoming fatal within one to seven days after infection.⁶

Both the chronic and acute forms of pulmonary avian aspergillosis usually present with respiratory symptoms such as dyspnea and respiratory distress characterized by gasping, accelerated breathing, and changes in vocalization, however they differ in other areas.^{4,5,6,7,9,10,12}

Firstly, acute aspergillosis is associated with a rapid onset of respiratory symptoms whereas chronic aspergillosis is associated with progressive development.^{5,6,7,10} Other symptoms for acute aspergillosis are excessive thirst, excessive urination, lethargy, weakness, vomiting, wing droop, stunted growth and development, ruffled feathers, and sudden death due to asphyxiation.^{5,6,7,10} Birds with acute aspergillosis can have anorexia however they are usually still in good body condition although emaciation and rapid weight loss may occur.^{5,6,9,10} In cases where infection disseminates to the brain there can be a loss of muscular function as well as neurological dysfunction such as twisting of the head into unusual positions.⁷ Finally, extension of lesions from the air sacs to the spine and sacral plexus can lead to paresis and paralysis.¹⁰ Symptoms for chronic aspergillosis include chronic weight loss leading to emaciation, wasting, and dehydration.^{5,6,7} While the symptoms for both chronic and acute aspergillosis can be caught in birds in captivity or those in rehabilitation, free living birds are usually found dead or dying, making it too late for treatment.⁵

Antemortem diagnosis of avian aspergillosis is difficult due to numerous factors, often depending on what diagnostic methods are available to the facility.^{5,8,9} Firstly, due to the nonspecific symptoms that usually develop when the disease is progressed, it is possible for aspergillosis to not even be considered when looking at patient history and clinical signs.^{5,10,12} Furthermore, there is no single diagnostic procedure that can definitively diagnose aspergillosis, requiring the use of several techniques.¹⁰ If a complete blood cell count is performed, it will show abnormalities associated with infection including an elevated white blood cell count.^{4,12} Radiography can be used to visualize potential lesions however they may not always be present, usually appearing only when the infection is severe, so lack of lesions does not rule out

aspergillosis.^{4,6,9,12} Serological assays such as enzyme linked immunosorbent assays for the fungal cell wall polysaccharides 1,3- β -D-glucan and galactomannan can be performed, however false negatives can occur in immunocompromised patients due to low levels of antibody production.^{9,10} If access to these diagnostic tests is not available, it can make diagnosis of avian aspergillosis difficult leading to worse patient outcomes. This is of particular concern for raptor rehabilitation programs and captive breeding programs where death of a single bird can have large consequences.

Confirmation of diagnosis requires identification of at least one *Aspergillus spp.* by culture and cytology or histopathology that shows hyphal growth with associated tissue damage.^{7,8,10} Samples for culture and histology can be obtained in several ways such as tracheal wash or lavage, and endoscopy.^{6,9,12} Endoscopy can also be used for visual confirmation of lesions inside of the air sacs and to determine the extent of infection.^{4,6,7,9,12} Histology is made using the periodic acid-Schiff stain or the Gomori methenamine silver stain which allow visualization of the fungus.⁹ Microscopic features seen on histology include hyphae with dichotomous branching and occasionally conidiophores if the sample was taken from the air sacs.⁹ Only culturing *Aspergillus spp.* without confirmation of lesions or a positive histology is not sufficient to diagnose aspergillosis due to the ubiquity of spores in most environments that can lead to false positives.⁵ Similarly, only seeing lesions is not sufficient because similar lesions can be caused by other fungal infections.⁵ Thus, both a positive culture and histology from a lesion is required for diagnosis.⁵

Because of the difficulties in diagnosing birds antemortem, most diagnoses of avian aspergillosis are made postmortem.⁸ Signs of infection will most commonly be seen in the lungs and the posterior (thoracic and abdominal) air sacs due to the pattern of air flow through the respiratory system.⁵ In early stages of the disease, lesions will appear hemorrhagic and swollen, however, as the infection advances, it progresses into granulomatous type inflammation.⁵ Similarly to the symptoms, macroscopic findings for chronic and acute aspergillosis differ from one another slightly. For cases of chronic aspergillosis, lesions of varying sizes, generally appearing as flattened yellow to white plaques with a cheesy appearance, can be seen in the lungs and air sacs.^{5,6,7,8,9,10} These plaques can be so abundant that they completely line an air sac.⁷ Sporulation and asexual reproduction of the fungus in chronic cases can lead to the development of a fungal mat in the air sacs that resembles bread mold.^{5,7,8,9,10} The color of this fungal mat can vary depending on the species of *Aspergillus* that caused the infection and can be used for species identification.⁵ For acute aspergillosis, there is usually a thickening of the air sacs, and the lungs are firm, dark red, and covered in small white to yellow nodules that are one to two millimeters in size.^{7,10} The lungs and air sacs may contain granulomatous nodules as well.¹⁰ Cheesy, yellow to white lesions in the air sacs can range from less than one millimeter to greater than fourty.^{5,6,8} Larger lesions may involve the serosa and parenchyma of one or multiple organs.^{5,8}

Treatment of avian pulmonary aspergillosis is done using antifungals. Because all of the available antifungals were developed for use in humans, there are no official recommendations for doses in birds.¹³ Dosage and regimens are instead based on individual experience, case reports, or values that are calculated from other species such as humans.¹³ In addition, due to the

difficulty in developing antifungal drugs that lack serious side effects in humans, there are limited options for treatment.⁸ Treatment in birds is usually done using amphotericin B (AmB) and triazoles.^{4,8,9} AmB kills fungi by binding to ergosterol, an important cell membrane component, and forming a pore in the cell membrane that leads to cell death.¹³ Due to molecular similarities between ergosterol and cholesterol, AmB is associated with high risk of renal and systemic toxicity.¹³ Because of this, the tolerability of treatment in birds is poor.¹³ It also has poor bioavailability when it is administered orally meaning that it has to be given intravenously.¹³ This can be difficult to do without using anesthesia or causing stress to the bird. Finally, there is an upward trend in resistance to AmB in isolates taken from avian species.¹³ For example, one study that took isolates from geese found that all 85 samples that were collected were resistant to AmB.¹³ This increase in resistance in combination with high risk of toxicity and the need to administer it intravenously limits the usefulness of AmB in treating aspergillosis in birds.

Triazoles function by inhibiting 14 α -lanosterol demethylase, an enzyme involved in the biosynthesis of ergosterol, which results in altered membrane function leading to cell death.^{8,13} The triazole drugs used for treating birds include itraconazole, voriconazole, and occasionally posaconazole.^{4,9,12} Itraconazole was the drug of choice for treating aspergillosis in birds before the introduction of voriconazole.¹³ When compared to voriconazole, itraconazole requires higher doses and longer regimens to achieve therapeutic levels in raptors which greatly increases the risk of hepatotoxicity.¹³ In addition, one study found that 21% of isolates from falcons were resistant to itraconazole.¹³ Voriconazole is a broad spectrum antifungal that has been shown to have higher efficacy than AmB and itraconazole for treating aspergillosis in mammals.^{5,13}

Therapeutic levels of voriconazole in raptors can be obtained one hour after administration compared to the several days needed for itraconazole.¹³ Side effects of voriconazole in birds are rare despite high frequency in humans and include depression, excessive urination, and liver dysfunction.¹³ Voriconazole also has numerous drug interactions that may exclude its use in certain patients.¹³ Though voriconazole has drug interactions and side effects, it has fewer side effects than AmB and itraconazole and is thus the preferred treatment for avian aspergillosis.^{5,8,13} Unfortunately, similar to AmB and itraconazole, strains of *A. fumigatus* recovered from domestic and wild birds have shown an acquired resistance against voriconazole, posing a risk to its use as a treatment for aspergillosis.¹³

Prevention of aspergillosis for birds in captivity or rehabilitation is easier and more effective than treatment and should thus be prioritized.^{6,12} The first method of prevention is environmental control via improving management conditions to limit the number of conidia present in the environment.^{5,9,13} This can include the removal of organic debris from enclosures, ensuring proper ventilation, avoiding overcrowding, and minimizing stress.^{5,6,9,10,13} Antifungal prophylaxis can be given to highly susceptible individuals as well.^{5,6,8,12} This is recommended for high risk birds in rehabilitation due to the high incidence of fungal disease in rehabilitation settings.⁸ In raptor rehabilitation this includes giving prophylaxis to newly admitted, severely injured, and ill birds.^{4,5,12} The preferred drug for prophylaxis is itraconazole administered orally although voriconazole can be used.^{4,12,13}

Because triazoles, namely voriconazole and itraconazole, are the drugs of choice for treating and preventing avian aspergillosis, there is a large concern surrounding the emergence of

azole resistant strains of *Aspergillus spp.* including *A. fumigatus*. Azole resistance is classified by high antifungal minimal inhibitory concentrations, meaning that greater dosages of the antifungal would need to be administered to inhibit fungal growth.⁸ At certain minimal inhibitory concentrations, it becomes unfeasible to increase doses as doing so greatly increases the risk of side effects and toxicity.¹³ Azole resistance was first reported in 1997 and has since been reported all over the world at increasing rates.⁸ One study in the UK showed that resistance rates in clinical isolates from humans rose from 0.43% to 2.2% between 1998 and 2017.⁸ Recent estimates for current rates of azole resistance vary between 0% and 26% depending on geographic location.⁸

Azoles inhibit fungal growth by targeting the enzyme 14 α -lanosterol demethylase (CYP51), preventing the conversion of lanosterol into ergosterol, an important component in the fungal cell membrane.^{8,13} Mutations in the gene for CYP51, *cyp51A*, have been correlated to the emergence of azole resistance in strains of *A. fumigatus*.⁸ These are often point mutations with or without base pair duplication in the gene's promoter sequence.⁸ Common point mutations associated with azole resistance cause amino acid substitutions of G54, G138, M220, and G448, causing impaired binding of azoles to CYP51, preventing their action in fungal cells.⁸ Mutations including duplications of base pairs in the promoter sequence of *cyp51A*, including TR₄₆/Y121F/T289A and TR₃₄/L98H, have been linked to azole resistance as well.⁸ These mutations lead to an upregulation of CYP51 and an increase in ergosterol synthesis, counteracting the effects of azole treatment.⁸ TR₄₆/Y121F/T289A is responsible for high levels of voriconazole resistance, while TR₃₄/L98H can cause pan-azole resistance.⁸ In addition to *cyp51A* mutants, one study found that 43% of resistant isolates did not contain mutations in *cyp51A* and

instead had mutations leading to overexpression of efflux pumps such as ATP-binding cassette transporters and the major facilitator transporter.⁸ Due to the already low success rates of treatment of avian aspergillosis, if an individual becomes infected with a resistant strain, the likelihood of survival falls even lower. Thus, the emergence of azole resistance is a concern in raptor rehabilitation and conservation.

There are two main origins of acquired azole resistance in strains of *A. fumigatus*. Firstly, the point mutations that occur in Cyp51A have been associated with long term azole treatment, including prior treatment of a fungal disease and prophylactic treatment.⁸ This is an issue in raptor rehabilitation due to the common use of prophylactic treatment for aspergillosis in high risk patients. Azole resistance has primarily been linked to the use of azole fungicides in agriculture.⁸ Fungicides are used to prevent the growth of *Aspergillus spp.* that are pathogenic to plants, such as *A. flavus*.⁸ These species commonly contaminate crops with mycotoxins that are carcinogenic and cause hepatotoxicity, teratogenicity, immunotoxicity, nephrotoxicity, and genotoxicity if consumed.⁸ Many of these fungicides have chemical similarities to the azoles that are used to treat aspergillosis.⁸ While *A. fumigatus* is not the target of agricultural fungicides, due to its ubiquity in nature, exposure to fungicides occurs frequently which promotes the selection for azole resistant strains.⁸ Additionally, azole fungicides do not biodegrade easily and have high chemical and photochemical stability causing them to persist in soil, water, and agricultural products.⁸ If contaminated food or water are consumed, it can cause exposure to azoles in humans and animals which can contribute to the development of azole resistance as well.⁸

While it is necessary to understand how azole resistance can negatively affect the outcomes for birds with avian aspergillosis, it is also important to view the interactions between birds and azole resistance from a One Health perspective. One Health considers health based on interactions between humans, animals, and the environment.⁸ Studies looking at strains with the TR₃₄/L98H and TR₄₆/Y121F/T289A mutations showed that those strains have low genetic diversity but global distribution suggesting that azole resistant strains can be spread.⁸ In fact, one study identified two strains with the same genotype that were isolated 7500 km apart from one another.⁸ It has been hypothesized that birds may contribute to this spread of azole resistance.^{5,8} Firstly, genomic studies have proven the ability of viruses to spread between environments via bird migration.⁸ Another study demonstrated that gulls are able to spread the fungus *Candida glabrata* via environmental contamination.⁸

Though it is unlikely that birds release *Aspergillus spp.* conidia during respiration due to the lack of a diaphragm to forcefully expel air, reproductive structures that produce conidia can grow in the air sacs of infected birds.⁸ This can lead to environmental contamination through feces, if the infection spreads to the gastrointestinal or urinary tracts, as well as through carcasses if a bird dies.⁸ Birds can also be exposed to stressors during migration that can predispose them to aspergillosis such as weight loss, fatigue, and food deprivation, increasing the likelihood that they carry a strain from one geographic location to another.⁸ These resistant strains can then go on to infect other birds, animals, or even humans. While this hypothesis has not been confirmed, it remains a concern as rates of azole resistance rise globally. This is of particular concern for human health as infection by an azole resistant strain can cause an increase in the already high mortality rates associated with the disease.

The Experience

Overall, I found my internship with Rocky Mountain Raptor Program incredibly rewarding. Prior to this experience, I had very little knowledge regarding raptors let alone birds, so being given the opportunity to learn more about them in such an in depth and up close manner was exciting. I personally enjoy learning as much as I can about diverse topics, so getting to take classes about various aspects of raptor biology and rehabilitation in combination with what I was learning from the internship itself was enjoyable. It was also fun to be able to work with wildlife and potentially have a positive impact on their conservation, which has always been a dream of mine.

As someone who is passionate about anatomy and physiology, one of my favorite parts of the internship was the opportunity to perform necropsies on birds that had passed away or had to be euthanized. I enjoyed performing the necropsies for multiple reasons. Firstly, I was able to feel and dissect fractures to better understand the healing process of bones. This understanding became applicable very quickly in my other internship with a vet clinic when an animal came in with a partially healed fracture that I was successfully able to palpate and describe. I was also able to use the necropsies as a way of practicing my avian anatomy by running through anatomical terms and locating specific anatomical differences between species. This information is important for me to practice as the anatomy of various animals including birds will be a major focus in vet school. Some of the necropsies enabled me to observe the effects that different pathologies have on animals. For example, there was one bird I performed a necropsy on that had a severe case of visceral gout impacting multiple organs that was likely the cause of death. The external exams on the birds gave me the opportunity to practice what I had learned in the raptor

identification class as I had to identify and age individual birds based on their physical features. Finally, performing the procedure of the necropsy with careful dissection provided me with skills that will translate into performing surgeries in my future career.

I also enjoyed being able to link my experiences from the internship with what I have been learning in my classes for both of my majors. Zoology and biomedical science do not have a lot of overlaps when it comes to classes at CSU, so it is rare that I get to combine both topics, but this internship allowed me to do that. While certain aspects of the internship applied to zoology such as caring for the raptors and learning about their natural history, behavior, and identification, there were others that also applied to biomedical science such as learning about the anatomy and physiology of raptors and diseases that impact them, performing necropsies, and providing medical care. I really enjoyed how this internship gave me the opportunity to experience and expand my knowledge on both topics that I was passionate about. In addition, it introduced me to the disease aspergillosis. I have a particular interest in zoonoses, and aspergillosis one that I was previously unaware of and enjoyed researching.

While my experience with this internship was positive, there are a few things that I wish I could have done differently. Firstly, I regret not asking more questions. Though I am a naturally curious person who is constantly wanting to learn about the world around me, I have issues with expressing that curiosity to others, which can make learning from experiences such as my internship more difficult. I did try to push myself to ask more questions, and I believe that I did a better job than I normally do in those situations, however, I still wish that I had asked more so I could have learned more about raptor rehabilitation and wildlife medicine. Secondly, I would

have preferred to push myself more by actively volunteering to help catch and assess birds or even administer medication. While I did get to do all of those things, I always waited to be asked to do it instead of seeking out the opportunities myself. I feel that if I had pushed myself to do so, I would have gained a lot more experience and knowledge from the internship.

The one thing that I wish most to be able to change is my health, which impacted my experience in numerous ways. Firstly, for the majority of the summer, I was taking long term immunosuppressants to manage my chronic illness, including a JAK inhibitor and high dose corticosteroids. Due to the immunosuppressive effects of these drugs, I had a greatly increased risk of infection. While this did not interfere with most of my responsibilities, out of an abundance of caution, it prevented me from being able to perform certain necropsies, namely those for birds that were suspected of having aspergillosis. As such, I was never given the opportunity to view in person what the manifestations of pulmonary aspergillosis in birds look like. Had my risk of infection not been a limiting factor, I would have been able to learn more about and experience a disease that I find fascinating. However, because I was missing out on some of the necropsies, I learned to take advantage of the ones that I was able to take part in. In each one that I performed, I tried to practice and apply some of the skills that I had learned both from the internship and from my classes.

In addition to the immunosuppressive drugs that I was taking, I unfortunately had to receive major surgery after only four weeks of the internship due to a decline in my health. Because of this surgery, I had to miss three weeks of the internship totaling to 60 hours of experience. During this recovery period, I missed out on opportunities such as a presentation by

the education team about the importance of public education in conservation, as well as releases of some of the birds that I had been working with. Not only did this hinder my learning experience, but also my life experience, both valuable things that I desired to gain from this internship.

Coming back from my initial recovery period, I felt behind on everything that I was supposed to be learning and doing as part of the internship. Coupled with the fact that I had physical restrictions that prevented me from performing certain activities such as catching and restraining birds, it was disheartening. It felt as though everything that I had planned for my summer and my future was falling apart because of something out of my control. Though the staff, volunteers, and other interns, did their best to support me after my surgery, in my haste to catch up on everything that I had missed, I let my curiosity fall to the background. I asked fewer questions about how and why different things were done and my focus shifted from active learning to trying my hardest to not feel so behind.

Conclusion

Through my internship with the Rocky Mountain Raptor Program's rehabilitation department, I was able to participate in a critical component of raptor conservation. Additionally, I was able to learn and expand my knowledge about raptors, their management, and aspects of their medical care such as various diseases that afflict them. One such disease that I learned the most about was avian aspergillosis, a fungal infection of the respiratory system that is a major concern in rehabilitation programs. Though there are a few things I wish that I could change from my experience with the internship, it was very fulfilling, educational, and fun overall. As I

continue to work with the rehabilitation team, now as a volunteer, I hope to continue learning about the processes involved in raptor rehabilitation, so that I may one day be able to apply what I have learned to my career.

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