Mycobacterial Infection of Waterfowl: The White-winged Wood Duck Story

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Mycobacterial organisms have been referred to as "ducks of the microbe world". This alludes to the fact that they thrive in the shallow waters of the soil water interface, but can also reflect the severe impact mycobacteria can have on waterfowl populations.

Waterfowl mycobacterial infections are generally caused by organisms of the mycobacterium-intracellulare complex, most commonly by *M. avian* serotypes 1, 2, and 3. Recently, an increasing number of mycobacterial isolates have been determined to be *Mycobacterium genovense*- in some surveys up to 70% of isolates. This evolving trend further complicates ante mortem diagnosis by requiring testing modalities to be sensitive to both *M. avian* and *M. genovense* in order to be effective screening tools.

Mycobacterial infections are characterized as chronic, debilitating diseases usually seen in individual birds rather than in epizootics. They are characterized by numerous caseous lesions in the GI tract and major organs. There are no pathognomonic signs, symptoms relate to target organ and degree of damage. Mycobacterial infections are relatively rare in wild waterfowl, with a 0.3%incidence rate, compared to 3.6% incidence rate in total wild bird populations. Incidence in captive collections can be much higher.

All waterfowl are potentially susceptible to mycobacterial infection, although ducks and swans are more represented than geese, probably due in large part to exposure differences. Because they feed in the favored growth environment of the organisms, dabbling ducks are also more likely to be infected than grazing ducks. Research carried out at WWT in England also highlighted the role of genetics in increasing mycobacterial susceptibility. This is exemplified by the high captive infection rate of white-winged wood ducks, partially due to captive specie genetic diversity of less than 65%. Genetic factors, in general, seem to increase susceptibility in sea ducks and perching ducks, although the susceptibilities within specific groups vary.

As with most diseases, stress plays a major role in determining which individuals within an exposed population will become chronically infected. Reproductive stresses, overcrowding, poor ventilation, substandard nutrition, and management decisions (i.e. pinioning) can greatly exacerbate the disease process.

Early diagnosis of this relatively common disease can prove difficult. Currently no "gold standard" test exists. Acid fast fecal staining, long held as the test of choice, is at best positive in only 10%-30% of positive avian TB cases. Shedding of organisms is intermittent, is dependent on the organ or tissue infected, and is affected by the immune status of the infected bird in question. Elevated white blood cell counts are often reported with marked monocyte shifts, although this is frequently

unreliable early in the disease. Blood changes can also drop off late in the course of the disease as well, corresponding to immune system collapse. Mycobacterial cultures are relatively specific but do not often show high sensitivity and require long incubation times for results. Serology has also been historically unrewarding, although in one study by Cromie and associates, an ELISA did prove predictive in the mid to later stages of disease in selected duck populations.

Radiology can be a beneficial diagnostic tool in certain cases. It can be used to assess organomegally as well as the bone changes that occur in approximately 10% of cases. As with most other tests however, they are not typically effective until the disease has significantly progressed. Finally, endoscopy and biopsy are utilized by some institutions for mycobacterial screening. Even this is limited in its absolute ability to declare birds negative and holds the inherent time/risk/cost implications. It is usually best reserved for positive diagnosis of suspected clinical cases. In all situations, diagnostic ability improves as the disease progresses and any birds showing wasting symptoms should be isolated from the collection and tested by multiple laboratory modalities.

The treatment of avian TB is controversial. Treatment can be attempted, but due to development of drug resistance, poor prognosis, and zoonotic concerns, is not generally recommended. In the special circumstance where treatment is deemed necessary, prolonged combination therapy is generally utilized. Examples of medications used include; isoniazid, ethambutol, rifampicin, rifabutin, azithromycin/clarithromycin, ciprofloxacin, and amikacin.

With the inherent diagnostic and treatment difficulties, control and prevention are the mainstays to collection protection. This problem is magnified by the prevalence and resistant nature of the organism(s) involved. Mycobacteria are ubiquitous organisms found routinely in most environments. They have been cultured in up to 40% of surface waters, and in nearly 100% of municipal water supplies. They thrive in a variety of environmental conditions and can persist even in seasonal sub-zero temperatures for up to 7 years. They are normal soil inhabitants and can persist protected underground and multiply in earthworm and amoeba hosts.

Compounding the problem, low level environmental exposure by waterfowl early in life has been found to decrease resistance to pathologic mycobacterial later in life. Also, once infected, waterfowl can shed huge numbers of organisms into the environment for long periods of time prior to displaying demonstrable clinical symptoms. This subclinical/environmental cycle enhances and easily perpetuates the epizootic conditions in many waterfowl collections.

Environmental disinfection is unfortunately not a viable solution to the problem. Mycobacteria, in particular M. avian, are notoriously resistant to most common disinfectants. Mycobacteria have routinely been shown to grow in chlorinated water (500 times as resistant as E. coli), and are resistant to ozone and most common safe disinfectants. Even disinfectants labeled as "tuberculocidal" cannot be depended on for field use. "Tuberculocidal" refers to organism destruction on non-porous surfaces with extended contact times at room temperatures. Natural surfaces are seldom non-porous and organic material, hard water, and lower temperatures will inactivate the disinfectant or require a much longer contact time than is feasible in the field. Hot water is also not the answer. Mycobacteria have been shown to thrive in hot water and will even grow in hospital hot water heaters. In addition, due to their adherent properties, they are difficult to physically wash off of contact surfaces making sanitation that much more difficult.

The two most commonly used veterinary "tuberculocidal" disinfectants are aldehydes and phenolics. Both are easily inactivated by organic material, have poor stability, and are tissue irritants. Unless used carefully according to strict dilution requirements, either can be potentially toxic or carcinogenic to the users and environmental inhabitants. Due to the inherent limitations

stated previously, neither has proven effective for mycobacterial elimination under field conditions.

Maintaining an alkaline soil, however, does show potential as a mycobacterial deterrent. A Johne's study (M. paratuberculosis) resulted in a ten-fold decrease in paratuberculosis cases after lime treatment of pasture soil increased the soil pH to over 7. This was not a controlled study and obviously more work is needed to determine successes and limitations, but this technique seems to hold promise to help minimize environmental build-up in exhibits. Our goal for waterfowl enclosures is to minimize the untreated soil areas and utilize the lime treatments in a manner to be effective without being injurious to the collection. Drying of infected soil alone was not found to be a successful mycobacterial deterrent.

UV light may be the most effective anti-mycobacteriocidal agent available for zoo collections. Testing has demonstrated complete disinfection in water suspensions at 15 mW-s/cm2. Hospital ventilation sanitation systems utilize 100 mW-s/cm2 UVC devices for total mycobacterial air-borne sanitation. UV light is only effective, however, with direct mycobacterial contact. Shaded areas or areas where organisms are shielded by soil will not be affected by UV radiation. At WWT one survey found a 55% higher mycobacterial infection rate in shaded pens as compared to pens directly exposed to sunlight. To be successful, enclosure design must incorporate the ability to minimize persistent shading (or rotate shaded areas), and eliminate the shading of soil-water interface areas.

Until screening and treatment methods radically improve, mycobacterial control is best accomplished by minimizing environmental contamination and minimizing stress on the bird collection. New birds coming into an existing collection should be screened by multiple modalities in a quarantine of appropriate length. We recommend a minimum of 60 day quarantine, although the length could be extended based on the type of bird, the source of bird, mycobacterial history of source, and results of quarantine testing. Since moving and quarantine represents a major stress to an individual bird, the ability to detect the organism by ante mortem diagnostics should be improved in these situations. It should also be realized, however, that if the destination site for the incoming bird is currently contaminated with higher than normal levels of mycobacteria, the stress of introduction could result in increased risk of new infection. It is therefore the responsibility of the receiving institution to provide a habitat as mycobacterially "friendly" as possible and not to subject the bird to higher risk factors than it faced in its previous environment.

It is important that future exhibit designs minimize mycobacterial "footholds". Allowing UV exposure to as much water and soil as possible, rotation of shade areas, elimination of soil/water interface, alkalizing soil, and eliminating stagnant water are a few tools to minimize environmental mycobacterial build-up.

Culling clinically ill or wasting birds before heavy shedding occurs is also an effective tool to maintain flock/collection health. The WWT at Slimbridge, England have taken this to an objective level. Using historical statistical analysis of their collection, the ages that individual species are most likely to begin shedding mycobacteria into the environment have been determined. These birds can then be removed from the environment before becoming clinically ill and before major shedding begins. This is an aggressive measure necessary to address a historically endemic situation, and may not be applicable to most waterfowl collections. It does, however, highlight the need for close population surveillance, and demonstrate the advantage to lowering environmental contamination.

The White-winged Wood Duck Story

White-winged wood ducks are large, tree perching, deep forest ducks native to Assam, Thailand, Sumatra, and Java. Known in some areas as the "Spirit duck" for their haunting vocalizations, they live solitarily, in pairs, or small groups that feed nocturnally in shallow still waters. Due primarily to habitat pressures, the numbers in the wild are critical. They are listed CITES I and represent the only anseriforme SSP. Approximately 40-60 individuals exist in the USA's captive program, down from the over 120 birds recommended by the white winged wood duck SSP. Mycobacterial disease has played a significant role in the numbers decline in captivity. Currently, breeding recommendations are in place for all institutions holding pairs and it is hoped to build numbers back up to stable levels within the near future.

Historically WWWD's have had severe mycobacterial problems in captivity. Up to 84% of individual deaths in certain collections have been linked to avian TB. These high rates in captivity may not accurately reflect the true sensitivity of the specie in the wild, as poor genetics and exposure factors play a role. Due to the limited number of founders of the captive WWWD population (7), the genetic diversity currently stands at approximately 63%. Research in other species has shown that this lack of genetic diversity can have severe impact on the immune status of the individual, predisposing to mycobacterial infections.

Husbandry failures have also played a major role in the WWWD's mycobacterial problems. A solitary, tree perching duck in the wild, where mycobacterial contact is minimal, is now most frequently kept as a pinioned, ground bird in large mixed groups. The resultant increase in stress on the individual would further minimize resistance to the mycobacterial organisms it now routinely contacts. Natural husbandry traits also work against WWWD's in captivity. Their method of dabble feeding at the soil-water interface in shaded areas places them in areas of highest mycobacterial concentrations in captivity. The introduction of the wild caught naïve founder population into a large mixed collection endemic with mycobacterial has had tragic results. Not only did it lead to large numbers of individual losses, but more importantly, it led to losses in an already small irreplaceable genetic pool. Furthermore, due to endemic mycobacterial levels at the initial captive propagation site, infected individuals may well have been unknowingly disbursed to other collections to perpetuate the mycobacterial cycle.

Currently, the WWWD SSP has undertaken several projects in an attempt to better understand and improve the health and genetic status of white-winged wood ducks in captivity. Quarantine and necropsy protocols have been developed and pilot projects are underway to determine genetic diversity and assess ante mortem avian TB screening methods.

Results will be presented on the mycobacterial screening project. This project has utilized serial screening of a pilot group of WWWD's to evaluate acid fast tests, two separate ELISA tests, a BELISA screen, and fecal PCR testing. As has been previously described, early diagnosis of mycobacterial infections is problematic, and unlikely to be resolved by any single test in the near future. Improved quarantine screening, environmental protection, and stress reduction will undoubtedly prove to be more productive and beneficial to the specie in the meantime.

Toward this end, the white-winged wood duck SSP is currently making husbandry recommendations aimed at minimizing mycobacterial exposure and resultant infections. These include:

1. Maintaining non-pinioned birds where possible.
2. Maintaining birds in breeding pairs or small non-breeding groups.
3. Minimizing temperature extremes (50-80 degrees F).
4. Minimizing soil-water interface on ponds.
5. Constructing ponds to be cleanable with moving water.
6. Minimizing shaded areas and allow rotation of shaded areas for periodic UV exposure where possible.
7. Routine health screening/aggressive work up on "wasting" and ill individuals.
8. Maximizing nutrition.
9. Encourage free flight exhibits with natural perching/nest boxes.
10. Exclude wild fly-ins.
11. Minimizing shaded soil areas and maintain soil pH at 7 or greater.

The SSP is also currently in the process of developing a propagation center to test natural husbandry techniques and minimize mycobacterial contamination. In addition, as previously stated, we are also attempting to better characterize the current genetic diversity of the captive held WWWD's through blood DNA analysis. Once this data is accumulated and analyzed, it is expected to allow improved breeding choices. It will also help the SSP to locate any potentially valuable genetic individuals to bolster the current population.

It is hoped, and anticipated, that achievable husbandry and genetic improvements can make demonstrable changes in the current and future captive SSP white-winged wood duck population. With improved diagnostic testing in the near future, we look forward to simplifying husbandry guidelines and minimizing the impact of individual disease entities on the captive population.

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