Investigating the Detection Methods of White-Nose Syndrome in Bats

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AP Research

April 2019

Word Count: 4384

Abstract

White-nose Syndrome (WNS) is a disease that kills bats during hibernation by attacking and eventually penetrating the skin to kill the bat. The disease has spread rapidly throughout the United States since 2008 when it was introduced from Europe. Bats have a mortality rate of almost 100% due to the fungus *Pseudogymnoascus destructans* using all of the energy a bat needs for hibernation leaving no energy for them to survive (Lorch et al., 2010). Scientists must detect the disease quickly to aid in determining a cure. There are three main detection methods which are ultraviolet light detection, Polymerase Chain Reaction (PCR) and histology testing. Histology testing is used today and is a control in this experiment. This paper uses systematic literature review to compare the detection methods. The databases used were Google Scholar, Wiley, PLoS and the California Lutheran University library databases. Currently, PCR is the most effective method as it has the highest accuracy of 94.2% of the methods tested and a total processing time of one hour.

Key Words

White-Nose Syndrome (WNS), Polymerase Chain Reaction (PCR), Ultraviolet Light Detection (UV light detection), *Pseudogymnoascus destructans* (Pd) .

Introduction

White-nose Syndrome (WNS) is a fungal infection called *Pseudogymnoascus destructans* (Pd) that occurs in bats when they hibernate. Pd isa psychrophilic fungus which means that it can only survive in cold environments (Micalizzi et al., 2017). The disease first appeared in the

United States in 2006 and it has killed over six million bats in the United States alone, creating the problem scientists in the biology field are trying to solve (Thapa et al., 2016).

The disease originated in Europe and has made its way from one cave in New York to caves all around the East Coast and the Midwest (Lorch et al., 2016). As of now, WNS has been confirmed in thirty one of the fifty United States. However, most of the cases of WNS in the United States are around Pennsylvania and the Tennessee area because there are many caves and the latitude is far enough north to get the cold temperatures needed for Pd to attack the bats during hibernation. WNS has spread rapidly, but most bats in Europe are immune to the disease due to their genetic mutations over time which leads experts to believe the cure is near in the future (Lučan et al., 2016).

Bats hibernate during the colder months from October to March and Pd penetrates into the bat's skin and prematurely wakes them up from hibernation, causing them to starve and die. The bats are unable to function in the winter because they are unequipped to hunt and protect themselves in the cold temperature and eventually die (Bouma, Carey & Kroese, 2010). When Pd comes in contact with a bat, it increases the amount of carbon dioxide in the blood of the bat. When a bat reaches the threshold for the amount of carbon dioxide it can handle while hibernating, it wakes up and begins a constant cycle of hyperventilating, losing water, losing more nutrients and electrolytes and wasting more energy. This is a constant cycle for about a week before the bat dies. Typically, a bat does not survive a full cycle and usually dies after losing water (Verant et al., 2014). Bats need to hibernate every winter to slow down their metabolism, heart rate and breathing rate, while lowering the body temperature that has slowly increased throughout the rest of the year from April to September (Zukal et al., 2016). This

makes them able to work in the warmer months to survive, but Pd interrupts the hibernation period early by waking them up prematurely. This kills the bats because they use all of their energy stored for hibernation to fight the disease (Bouma et al., 2010).

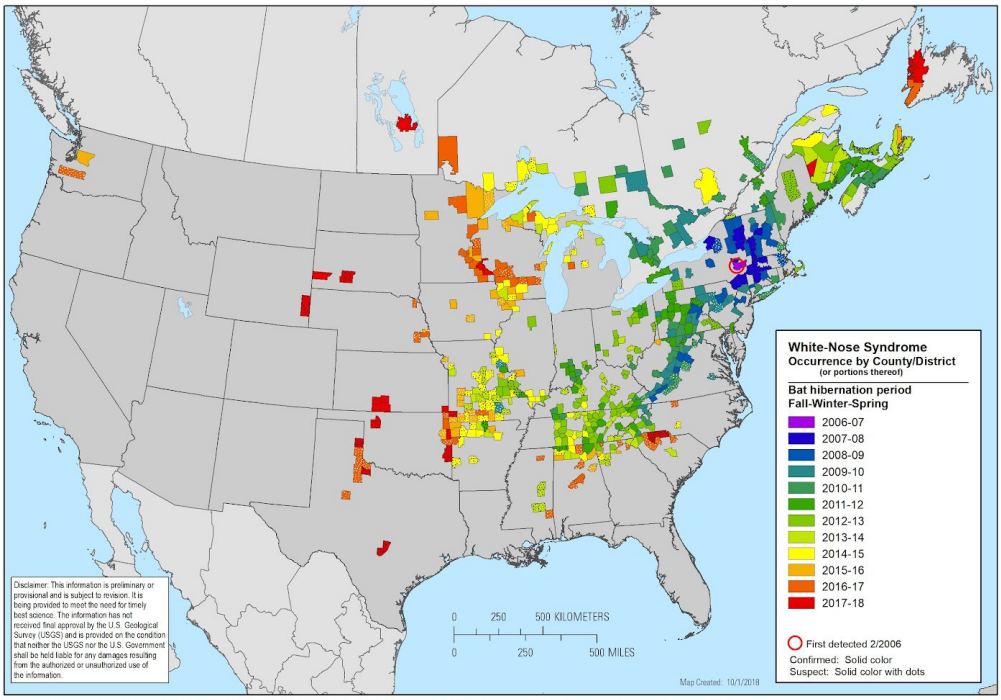

Fig. 1: This is a map of the spread of White-Nose Syndrome in the United States. It was introduced in 2006 and has advanced west across the United States (White-Nose Syndrome Foundation, 2018).

Bats Significance

Currently, there is not a cure for WNS to prevent bats from dying due to the current mortality rate that is above ninety-nine percent (Jonasson & Willis, 2011). WNS has killed approximately 6,700,000 bats in the United States alone since the disease first emerged in 2006 in New York (Frick et al., 2010). Since a bat can eat up to a thousand bugs, such as moths, beetles, and flies, a night and a colony can eat a million bugs per night, they are crucial to the

food chain (Karp & Daily, 2013). Bats are in the middle of the food chain, being hunted by cats, racoons, and hawks while eating mosquitoes and moths. Without their presence, the food chain would become unbalanced and the animals hunting them would die. In addition, bats save humans an average of twenty three billion dollars a year on insect control, for farms and homes (Law et al., 2018). Bats are able to do this by being a natural insect control, eating bugs and insects which are trying to eat crops. Without bats, a farmer would have to use a pesticide which can be costly and potentially damaging to their crops due to the chemicals.

Name of WNS

The name White-Nose Syndrome came about because of Pd spreading to a bat's nose and turning it white, indicating that the bat is infected with WNS (Trivedi et al., 2014). As of right now, WNS is the only disease that is associated with turning turn a bat's nose white, so if a bat has a white nose, it has WNS. Another characteristic of WNS is that the rest of the bat looks normal and healthy when it is newly infected with WNS, so the nose is the only indicator that a bat is sick. The nose color emerges right before the bat dies, which is too late to receive a treatment.

***Pseudogymnoascus Destructans* (Pd)**

WNS is spread from Pd attaching to human clothing and traveling with it to the person's next destination (Ingersoll et al., 2016). Therefore, the same clothing a person wears inside in a cave should never be worn to another cave, even if the clothing has been washed and the visits are years apart. For example, if a tourist visits a cave in Tennessee in 2004 and then visits a cave in New York in 2013, they cannot wear the same clothes as they wore in 2004 because the fungus can still be attached to their clothes. At almost every cave with a visitor center and full

time park rangers, visitors will be educated on this policy and not permit anyone in the cave that is wearing the same clothes as were worn in a previous cave visit. Scientists have not found a cure to prevent the fungus from getting on clothes yet, but are working to find one soon (Reynolds & Barton, 2014).

Poly Chain Rapidmerase (PCR)

A method used to detect WNS is Poly Chain Rapidmerase, PCR, as a way to detect Pd on the bat within a couple of hours (Lorch et al., 2010). PCR has three main steps to amplify DNA that can be viewed under a microscope easier in order to determine if Pd is on the bat. First, denaturation occurs when the DNA is heated and splits into two separate strands, then the temperature is lowered in annealing to allow the DNA primer to attach to the template DNA. Finally, the template DNA is separated into two identical strands. This process can be repeated as many times as you need to get the desired amount of strands of amplified DNA (Wellcome Genome Campus).

Fig. 2: This image depicts a bat being tested for White-Nose Syndrome. It is being held for the scientist to extract saliva for a PCR Test. The saliva will later be tested for the fungus Pd.

(University of Illinois, Steve Taylor).

PCR can be processed within hours of the samples being collected which can lead to more bats being screened in a short amount of time. Also, the fast results can lead to treating the bat sooner. The average turnaround time from the bat being tested to getting a result is between one and three hours, depending on the scientist and the amount of times they replicated the DNA strand (Lorch et al., 2010). Most scientists amplify the DNA by PCR once and if the results are clear, then they stop. However, if the results are unclear, then they perform PCR again with the original DNA strand to get more copies of the strand to try to make a conclusion. The more times it is replicated, the longer it takes.

UV Light Detection

UV light detection is another detection method being researched in this paper. Scientists look at bat's wings under UV light because the light makes Pd glow a fluorescence yellow orangish color if Pd is present on the bat (Mascuch et al., 2015). That glow can be captured with a traditional camera, even a cellphone, so the cameras being used are not unique or expensive. UV light photography produces a picture, that can be inputted in a computer to quantify the amount of infection using UV light and analytical software to measure the amount of brightness on a wing. However, most scientists can look quickly at the photograph on a camera and see whether there is a fluorescent color on the bat’s wing.

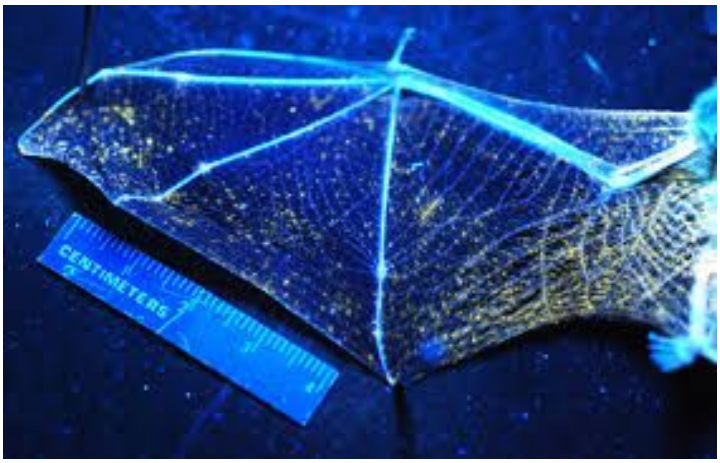

Fig. 3: This picture shows the testing of UV Light Photography on a bat's wings. The bat has fluorescence spots on its wings which signals the presence of Pd. on the bat (Turner et al., 2014).

The overall cost of UV light detection is inexpensive, but the paper placed over the bat is expensive. The UV wavelength used in the paper causes mammal cells infected with the fungus to glow. Most scientists conducting this test used a standard Nikon Camera DSLR mounted on a tripod over a UV box to zoom and shoot the photos (Lorch et al., 2010). Mounting the camera on a tripod is necessary on a to obtain clear photos since UV screening requires darkness and long exposure times. There are many other things such as various other fungi, bacteria, minerals, semen, etc., that could appear fluorescent when a bat is being tested for WNS.

Histology

Histology testing is the control group in this paper, as most scientists use this method today to determine if a bat has WNS. However, the long turnaround time associated with this method is not helpful to scientists since by the time a result is concluded, the bat has died of WNS already (Lorch et al., 2010). Scientists are discovering the need to come up with a better method to detect WNS earlier in a bat. Histology is performed by creating a lesion and removing

tissue and then examining the section of the tissue under a microscope. Turnaround time for these results can take weeks but the tests are always accurate based on the effectiveness of testing a whole piece of tissue. The process was invented in the 1660s and has been the normal standard test for detecting WNS and other diseases on animals and humans due to its accuracy (Meteyer, 2009). Normally, a bat must get tissue removed from three to five square centimeters of the bat's skin on its wing in order to view the tissue under the microscope to get the result needed. Many are now questioning the invasiveness of histology testing because some bats are very small and missing a tissue chunk can prevent them from flying at their normal ability. Another concern is the bat contracting a new disease in the time that it is being operated on due to the open wound in the wing. All scientists and researchers must have a license to handle bats to get a tissue sample. Because of the invasive process needed, the bat can experience mortality even if it does not have Pd due to the detection procedure itself (Meteyer, 2009).

Also, bats who survive WNS infection can become reinfected. Studies are underway to try to explain differences in the susceptibility of different species and the species ability to resist WNS. In addition, it is important to be aware that there are laws that protect bats and there are health risks associated with handling bats (Kovacova, 2018). The scientists at the NWHC that handle bats are vaccinated for rabies and take special precautions to prevent disease transmission. They also make sure that their colleagues in the field have permits that allow them to handle and collect bats.

Overall, bat populations are decreasing rapidly due to the spread of WNS in the United States. Bats are not being detected early enough to receive treatment and with the debilitating disease, bats are killed normally within a week of contracting WNS. With human traffic, WNS

has been confirmed in over half of the states in the United States. As of now the main detection methods for WNS that detect Pd from bat wings are as follows: UV light detection, PCR, and histology. These methods can try to ensure a bat can receive treatment to survive. Bats are paramount to the food chain and humans, saving billions of dollars a year and controlling the regulation of other animals in the same environment, so a fast but accurate detection method is needed to help save the bat population in the United States.

Purpose

The purpose of this study was to investigate detection methods of White-Nose Syndrome, looking specifically into UV-light detection and PCR analysis. By investigating these newer detection methods, scientists will be able to detect WNS before the disease spreads further into the bat's skin so the probability of survival will be increased because detecting the disease earlier can increase the chance of survival. Currently, the method being used, histology, takes too long to give its results so bats are already dead by the time they are able to obtain the result, prompting scientists and researchers to seek to find a new method that is as accurate as the old method but faster in turnaround time. The accuracy of the methods will also be investigated as the method that scientists will use in the future must be reliable to diagnose a bat correctly.

Research Question

What is the most reliable, fastest detection method to detect WNS in bats?

Hypothesis

Alternative: The UV light detection is less effective than PCR due to the uncertainty of the reflection of the fungus on the bats wings.

Null: The UV light detection is as or more effective as PCR in detecting WNS.

Methods

This analysis will be using the method of systematic literature review to get data from papers that are already published by universities and agencies through various databases such as PLoS ONE, ScienceDirect, Springer, Ebscohost, Google Scholar and the California Lutheran University library databases. Some key words used to find relevant articles were "white-nose syndrome," "fungal disease in bats," "Pseudogymnoascus destructans," "disease in bats," "Ultraviolet Photography," "rapid chain polymerase testing," "detecting white nose syndrome," "fungal disease detection," "bats hibernation," and "detection methods of bats."

The data range or time-period for the data collection sources was from 2008 to 2018. The disease emerged in 2006 so the newest detection methods were developed and tested starting in 2008. However, some background information about bats and their effect on the environment and other species ranges from 2000-2018 due to the high volume of articles on the effect of bats have on farms' insect control. These articles contain information specific to the detection methods, and provided a strong foundation to the topic, so they have been utilized for background.

The systematic literature review was the most appropriate method for this study because it allowed the data and conclusions from many previous studies to weigh in to the solution. It would not be possible to collect data myself because of necessary permits and regulations

prohibiting the independent data use; so systematic literature review was the most effective way to get data to reach a conclusion. Furthermore, surveys are not relevant because an opinion on the spread of WNS and how to detect it is not helpful due to the lack of specialized knowledge. Secondary data analysis would also not work because there is not one agency or source with the necessary data to compile a true conclusion. Many companies are biased towards their product or solution, which can create inaccurate results.

At the beginning of this research, there were about forty five papers read, mostly about background on the topic and going briefly into the different subtopics of WNS and around four were able to be used on this project directly for data or more specific background on the detection methods of WNS. When papers were first examined, it was common that they mostly studied the spread of WNS and how that resulted in the mortality rate, depending on the cave. This prompted the use of UV light as a possible cure and a diagnostic method, resulting in comparing multiple diagnostic methods. There have also been studies on PCR and its wide use in WNS, so the comparison came naturally. Then, around twenty five more papers were found to try to get more data to be able to find a trend within the numbers. I was able to extract data from nine papers to come up with my conclusion.

The data collected was originally recorded in data tables using Google Sheets. Then, this data was transferred to Microsoft Excel to perform statistical analysis and the creation of charts, as Google Sheets was missing statistical functions and the ability to make certain graphs. Utilizing Excel, one bar graph was made to show the differences in the amount of false positives and negatives between the values. Also, standard deviation was calculated with Excel.

Results

Table 1: This data table shows the accuracy of UV light detection compared to histology. As recorded, the UV light detection was able to correctly identify a bat as positive or negative every time except with *Myotis lucifugus* because it has one false negative and one false positive. Other than one error in a false negative and false positive, UV light detection is accurate.

United States Species	**Positive**		**Negative**		**Total**
	UV light detection	Histology	UV light detection	Histology	
Myotis lucifugus	59	58	40	41	99
Eptesicus fuscus	1	1	1	1	2
Myotis leibii	1	1	0	0	1
Myotis septentrionalis	5	5	7	7	12
Perimyotis subflavus	11	11	16	16	27
Myotis grisescens	0	0	7	7	7
Myotis velifer	0	0	11	11	11
Myotis sodalis	0	0	1	1	1
Myotis yumanensis	0	0	1	1	1
Myotis austroriparius	0	0	3	3	3
Tadarida brasiliensis	0	0	1	1	1
Unidentified myotis sp.	3	3	0	0	3
Total	80	79	88	89	168

Table 2: This data table shows the detection of Pd using PCR in WNS with a 96% accuracy. The researchers tested the histology of the bat by using blood and tissue samples which is universally correct and compared those results with the PCR detection, concluding that PCR had two false negatives and no false positives with the overall seventy eight bats that were tested. These results were confirmed by histology.

Method	WNS Positive	WNS Negative	False Positive	False Negative	Diagnostic Sensitivity (%)
PCR	46	32	0	2	96

Table 3: This table compares the difference between UV light detection, PCR and histology. The amount tested were in a cave and the controls were tested in a lab. PCR has the highest sensitivity with one hundred percent accuracy in both the normal and control test. Next, the UV light had a lower accuracy of seventy three percent in the cave and eighty two percent in the lab. The PCR method also had more trials done than the UV light detection which proves to be a reliable method more times.

Method	Amount Tested (Controls)	False Positives	False Negatives	Sensitivity
UV light detection	55(48)	0(0)	15(8)	73% (82%)
PCR	61(47)	0(0)	0(0)	100% (100%)

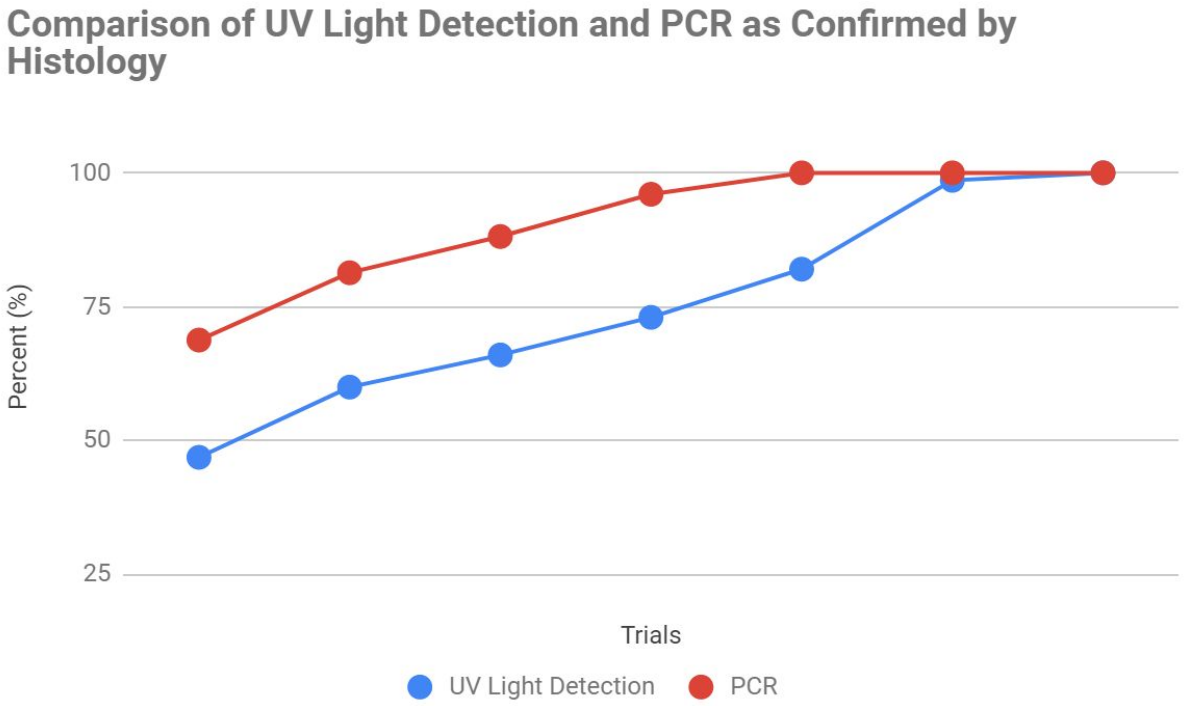

Fig. 4: This graph shows the different average accuracies for both PCR and UV light detection as compared to histology. UV light detection has a best-fit equation of $y = 9.0282x + 39.083$ and an $R^2 = 0.9772$ of which means the different accuracies are almost in a linear equation. PCR has a best-fit equation of $y = 5.1068x + 70.17$ and an $R^2 = 0.8453$ which means they are almost equal to being a linear function. The PCR is above the UV light detection for the majority of this analysis, showing it is on average more accurate than the UV light detection. Also, the UV light has some perfect accurate studies but also has some under fifty percent accurate, which can be inaccurate to scientists, depending on the conditions.

Discussion

Table 1 shows the detection of various fungi using fluorescence in WNS which was 98.9% accurate. The researchers tested the histology of the bat by using blood and tissue samples which is universally considered correct and compared those results with the fluorescence detection. In many of the species tested, the accuracy of UV light detection was one hundred percent perfect which is encouraging for the cure. The difference of one false positive test reading and three negative test readings is not that large due to the overall success of this method. The accuracy rate of 98.9% was almost perfect. Without Myotis lucifugus, UV light was a 100% accurate reliable method to locate Pd. Throughout the data table, there is only one difference between the fluorescence testing and the histology testing. There is one error for a false positive and missing a negative during the whole study. Other than one error, the tests are perfect in detecting the fungus Pd on bats. Histology takes a long time for the tests to get results so Fluorescence testing the UV light is a way to ensure the results are quicker. The data is

accurate due to the completed tests having only having one error. This is not too concerning l because there are false tests always in science so the one difference still deems UV light detection a viable detection method for detecting WNS. The change in one can be improved by having more tests done. In the second data table, the sensitivity for PCR is significantly higher than histology based on the amount of false negatives only being two out of thirty two. Histology had a much higher number of false positives having twenty two out of fifty two which is almost half of them being incorrectly counted for at an accuracy of around forty two percent.

This data shows that the PCR detection method was the most accurate between PCR and UV light detection methods. With the average accuracy of 94.2%, it is the most accurate compared to the average accuracy of 79.9%. That difference is huge because when twenty bats from one cave are tested, the PCR on average will have one mistake while UV light would have four false readings. Those readings determine whether the bat can get treatment and slow down the effects of WNS or die soon after. However, in another study as shown in table three, both methods did not have any false positive readings. This is most likely because of the Pd and its distinctive properties that make it easier to see through UV light detection and PCR.

In addition to the accuracy tests, PCR takes about an hour to three hours to get results back for the bat and requires a lab to test. The lab required must have a machine equipped to properly read a PCR analysis but such software and tables can be taken into the field to properly test on site. UV light detection does not require a lab to test the bats and results are instantaneous and can be seen within the minute. This can be helpful when looking through hundreds of bats and quickly determining whether they are infected or not.

Histology testing is used today widespread and is the control used in this research because scientists have determined that it is one hundred percent accurate. Scientists use a large section of a bat's wing which contains DNA and tissue sampling. The problem is that it takes a long time and is quickly being avoided in many tests today. The long turnaround time allows the Pd on the bat to expand eventually to the nose which determines the name WNS and could kill the bat. Simultaneously, the bat could fly to another cave and spread the disease. PCR allows for the disease to be caught early so the bat can be further tested for either treatment or to look for a way to track the disease. PCR only requires a swab test to be used to get a saliva sample and the UV light detection requires using the whole wing but the only restriction is to be layered with paper on top.

The PCR data is available more widespread than UV light detection because of the popularity and the knowledge of how to use it in other areas of the biology field. UV light detection has only been used for WNS and PCR is available to use for other diseases. Some scientists consider UV light detection a screening tool rather than a definitive diagnostic test for WNS because of the uncertainty of Pd producing fluorescence observed on the bats skin. It has been used to test for cancer in humans and other diseases in animals and microorganisms. The PCR test is used simply to amplify the DNA but not give definitive results to whether a bat is positive or negative. Scientists must determine that on their own. UV light detection is also known as a diagnostic tool but not a definitive test to calculate whether a bat is negative or positively affected by WNS. The cost for the paper to perform UV light detection is on a per use basis which is less cost effective than the PCR which can be done as many times needed for the

same cost. The only cost needed is the machine and the staff to interpret the results and run the tests.

Limitations

Due to limited papers available to the public, the number of articles included in this systematic review study is small, causing the data to be not widely spread. Furthermore, the majority of the data was from the same team of researchers from the U.S. Forest Service which offers a niche outlook. With most of the research being done within the time period of 2010 to 2011, the disease could have been spread to different parts of the U.S. Also, the majority of the tests only tested either PCR or UV light detection so the results cannot be taken directly but must have some error built into them. In addition, the tests completed were from different caves so the results may have varied slightly based on the fungus specifically being mutated from caves in the U.S. and the Czech Republic.

Conclusion

At the moment, the UV light detection is the best detection method to get scientists the most reliable and quickest results. The UV light detection was successful in detecting the WNS 86% of the time. PCR detection was accurate 96% of the time which is less reliable than UV light detection. In addition, UV light has the turn around time of results within a minute and PCR has the turn around time of within an hour. When scientists are testing many bats at a time, the quicker turnaround is paramount. PCR accuracy is better but the turnaround time is unappealing compared to the minute turnaround from the UV light detection. However, accuracy is more

important than the turnaround time being slightly longer. WNS is spreading rapidly west to states such as Washington and Oregon caves. The fungus P. Destructans is attaching to more clothes and is traveling throughout more locations in the United States and the cure has yet to be developed.. PCR can help find the disease early on a bat with certainty and provide time for scientists to come up with a reliable cure for WNS.

Acknowledgements

Thank you to Dr. Natalia Martinkova who graduated with a bachelors and masters degree from Comenius University Bratislava in Zoology. She completed her Ph.D. study at Charles University Prague with a specialty in zoology. She now is a full-time researcher and works at the Institute of Vertebrate Biology Academy of Sciences in the Czech Republic. Next, thanks to Dr. Anne Ballmann who is the Veterinary Medical Officer at the United States Geological Survey's National Wildlife Health Center. She earned her bachelor's degree in Biology at Northeast Missouri State University-Kirksville, then obtained her master's degree at the University of Tennessee-Knoxville and finally did her Ph.D. in Comparative Biomedical Sciences-Population Medicine at North Carolina State University. Additionally, thank you to Dr. Malhotra who got a Ph.D. in Cell Biology/Physiology from the University of British Columbia and is the teacher of AP Research STEM at Thousand Oaks High School.

Further Work

To continue this project, more data could be collected from other projects similar to the accuracy of the diagnosis methods. Also, studying the culture method, which could be used as

another alternative to detecting WNS, would be necessary to investigate all of the possible techniques to detecting WNS in bats. The recurrence rate of WNS infecting bats a winter later could be investigated because becoming reinfected could mean that the results found are false or have an error in them, not fully tracking the Pd on the bat. There are also smaller funguses that grow on the bat as a result of WNS which include Genomeces Destructans and investigating the detection methods for that fungus would be necessary to understand the whole picture of WNS in bats.

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