Investigating the Detection Methods of White-Nose Syndrome in Bats

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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..

Introduction

White-nose Syndrome (WNS) is a fungal infection from the fungus called *Pseudogymnoascus destructans* (Pd) that occurs in bats when they hibernate. Pd is a 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). 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.

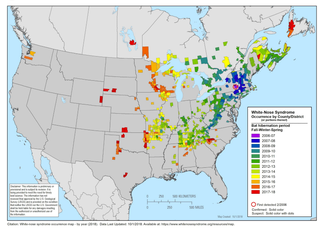

Fig. 1: This is a map of the spread of White-Nose Syndrome in the United States. It was introduced in 2006 and has advanced west across the United States (White-Nose Syndrome Foundation, 2018).

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). 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. 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. 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).

Fig. 2: This bat is being swabbed for saliva to perform PCR (University of Illinois, Steve Taylor).

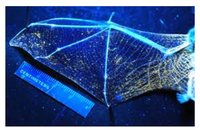

Fig. 3: This image depicts the presence of Pd, while performing UV light detection (Turner et al., 2014).

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.

Research Question

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

Hypotheses

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.

Discussion

Table 1 shows the detection of various fungi using fluorescence in WNS which was 98.9% accurate. 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. 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%. 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. 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. 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.

Methods

Systematic Literature Review
--Sources: C.L.U. databases, PLoS, Google Scholar, Wiley
--Keywords: White-Nose Syndrome, Detecting WNS, UV light detection, PCR on bats

Conclusion

At the moment, the PCR is the best detection method to get scientists the most reliable and quickest results. PCR detection was accurate 96% of the time which is more reliable than UV light detection with a 86% accuracy. Despite UV light detection having the turn around time of results within a minute and PCR has the turnaround time of within an hour, the accuracy of PCR outweighs the slow turnaround time.

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. 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.

Results

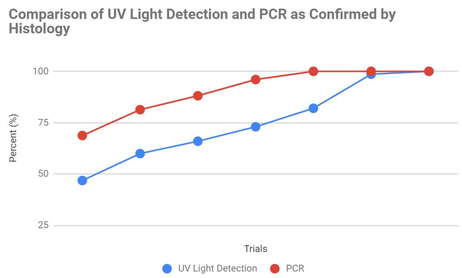

Fig. 4: This graph shows the average accuracies throughout the data collected in this research, with PCR being more accurate almost all the time except when both UV light detection and PCR are both perfectly accurate. UV light detection has a best-fit equation of $y = 9.0282x + 39.083$ and an $R^2 = 0.9772$ 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.

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 1: This table shows the accuracy of UV light detection as confirmed by histology, finding only one error as a false positive and negative throughout the study.

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%)

Table 2: This shows the accuracies when tested in the same environment.

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