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Department of Biology

Detection of Urine-based Deer Lures To Mitigate CWD Transmission in Pennsylvania

Ken Strauser, Aaron M. Haines and Richard Palmer

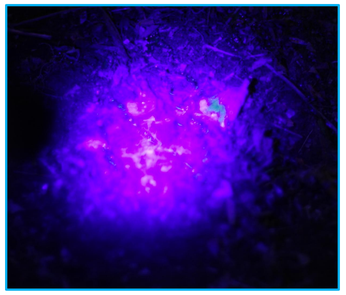
INTRODUCTION

Chronic Wasting Disease (CWD) is designated as a "dangerous transmissible disease" believed to be caused by prions, which are transmitted via bodily fluids of white-tailed deer (*Odocoileus virginianus*). There are no known treatments for CWD infections and the spread of this disease has a potentially severe detrimental impact on wild and captive white-tailed deer. Pennsylvania detected the first positive test for chronic wasting disease in a captive held whitetail deer in the summer of 2012. Since the identification of a pen-raised white-tailed deer infected with CWD was found in Pennsylvania, a Disease Management Area (DMA) was established around the area where the infected deer was located in Adams and York Counties. The Pennsylvania Game Commission established the DMA due to concerns that CWD may spread to wild white-tailed deer herd populations in Pennsylvania.

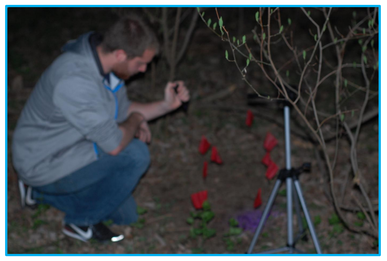
An executive order given by the Pennsylvania Game Commission established several restrictions within the DMA, one of which is the prohibition of the possession and of urine-based deer lures within the DMA. Urine-based deer lures are used by white-tailed deer hunters to attract deer into designated areas for harvest. It is estimated that from 10-40% of Pennsylvania hunters have been using white-tailed urine-based deer lures to hunt deer. At the same time commercial lures to attract white–tailed deer have become readily available to consumers at common retail sporting goods stores. However, urine-based deer lures have been known to be collected from domestic white-tailed deer herds, with the potential of an animal infected with CWD residing within these domestic herds. This can lead to the risk of urine-based deer lures containing prions that may cause CWD to be spread to wild populations of white-tailed deer via hunter use. Thus, the use of urine-based deer lures is prohibited within the DMA.

Due to this risk, the Pennsylvania Game Commission contacted Millersville University to try and determine whether urine-based deer lures can be identified in the field using chemical forensic tests. The objective of this study was to determine if there are reliable field based tests to enforce the current prohibition of urine-based deer lures in the DMA, and as part of the consideration for expanding that prohibition to all of Pennsylvania as part of a CWD prevention strategy.

We used the following forensics test kits to test for the presence of urine-based deer lures: Nite-site™ luminol kit (detects blood in urine), Uritrace® (detects human urine) and Urine Stain Ultraviolet Light (used to detect cat and dog urine). Our null hypothesis states that none of the 3 forensics tests will be able to detect the presence of urine-based deer lures.



Detection of a fresh urine scrape of Trail's End® #307 (glows blue-green when still wet) using Urine Stain Ultraviolet Light (UV).



Ken Strauser using a Urine Stain Ultraviolet Light (UV) to check scrapes for presence of urine-based deer lures.

METHODS

All analysis took place in the Applied Conservation Lab on the Millersville campus in Caputo Hall 116. Our field site was located on the Millersville University outdoor biological research park referred to as the 'Bush' located off Creek Drive on the Millersville Campus. Each of the 3 forensics tests (Nite-Site™ luminol, Uritrace®, and Urine Stain Ultraviolet Light [UV]) were tested for the following urine-based deer lures: Code Blue Doe Urine®, Code Blue Buck Urine®, Code Red Buck Urine®, Code Blue Scrape Mate®, Hunter's Specialties Primetime Doe Urine®, Wildlife Research Active-Scrape®, Wildlife Research Doe In Estrus®, and Wildlife Research Trail's End #307®. All forensics tests were used to test each liquid lure. Tests in the lab used water as the negative control and depending on the test, a positive control was used. Positive controls included human urine for the Uritrace® test and the UV tests, and bovine blood for the Nite-Site™ luminol test.

Uritrace® is specifically designed to test for presence of creatinine, a byproduct of muscle metabolism, found in urine. This test is common in forensics and crime scene settings and uses a small device into which 3 to 4 drops of the urine sample (wet, dry, stain) is placed. One well holds the urine sample and the other well holds a negative control, water. The well with the urine sample is compared to the color of the well containing water and a positive result is indicated by change in color intensity in the urine sample. This test was conducted directly for each urine-based deer lure and compared to water and human urine.

The Nite-Site™ luminol test is used in forensics to detect blood and glows in its presence in complete dark. It combines 5-amino-2, 3-dihydro-1, 4-pthalazinedione free acid, sodium carbonate, and sodium perborate tetra hydrate and these react with the blood heme to produce a blue glow. This kit was used to detect presence of blood in all the urine-based lure samples. About 2ml of each of the eight urine samples was poured onto separate 111mm watch glasses, along with bovine blood as a positive control and water as a negative control. Once the Nite-Site™ kit was prepared, this solution was sprayed onto the watch glasses and monitored for presence of a blue glow.

The Urine Stain Ultraviolet Light test utilized an LED UV flashlight, that was shone on eight ~2ml samples in 111mm watch glasses. Samples were assigned a number on a 0 to 3 scale, 0 being no glow and 3 being the most intense glow. In a second test, each of the urine types were then poured on one sheet of untreated cotton and tested for UV glow. In a third UV test, urine types were then blotted onto leather boots and tested with the UV light. A fourth UV test was conducted outside in the Millersville 'Bush' at dusk and the eight urine samples were poured on the ground in a simulated scrape. The light was immediately shone on the samples. After 24 hours, the samples in the scrape were tested again.

Both the Uritrace® test and UV light test were also conducted on 100% cotton t-shirts. Each urine-based lure was sprayed 4 times on 4x4" squares of fabric and left to dry for 24 hours and analyzed using UV light. A 0.25cm^2 square was also cut from the center of each stained fabric and soaked in100µl of distilled water for 1.5 hours. The water was then extracting using a 200µl pipet and dropped directly into the sample well on the Uritrace® device, for each urine sample.

RESULTS & DISCUSSION

The Uritrace® test did detect human urine as is designed, and also detected a few of the deer urine samples (Table 1). The human urine gave a deep red color in the test. The closest to the positive control was the Code Blue® Buck urine, which yielded a less intense red-orange. Code Blue® Scrape Mate gave off an orange color. Code Red® Buck urine and Primetime® Natural Doe gave a yellow-orange color. The rest (Code Blue® Doe, Active-Scrape®, Doe In Estrus®, Trail's End® #307, and the negative control, water) yielded a negative result, giving no change in color. The variation in colors could be correlated with how diluted each urine type is and further testing would be required. The 100% cotton t-shirt extraction for urine yielded all negatives after extracting the sample for Uritrace® testing (Table 1).

Urine Type	Uritrace®	UV	Nite-Site™	Uritrace® Shirt Extraction	UV on Cotton[1]	UV on Shoe Leather	UV Scrape[2]
Code Blue Doe	Yellow	1	No glow	Yellow	0	0	0
Code Red Buck	Yellow-Orange	1	No glow	Yellow	0	0	0
Primetime Doe	Yellow-Orange	1	No glow	Yellow	0	0	0
Code Blue Scrape-Mate	Orange	2	No glow	Yellow	2	0	1
Active-Scrape	Yellow	2	No glow	Yellow	2	0	1
Doe In Estrus	Yellow	3	No glow	Yellow	1	0	1
Code Blue Buck	Red-Orange	2	No glow	Yellow	2	0	1
Trail's End #307	Yellow	2	No glow	Yellow	2	0	2
Water	Yellow	0	No glow	Yellow	0	0	0
Human Urine	Red	1	No glow	N/A	N/A	N/A	N/A
Bovine Blood	N/A	N/A	Blue glow	N/A	N/A	N/A	N/A

Table 1. Compiled results of tests currently completed. A positive for the Uritrace ® is any color darker than yellow and a negative is yellow. In the Urine Stain Ultraviolet Light (UV) test, 0 means no glow and 3 is the most intense glow. In the Nite-Site™ test, No Glow yields a negative and Blue Glow yields a positive, or presence of blood. [1] Untreated Cotton. [2] Cleared area on the forest floor.

The Nite-Site™ test demonstrated that there is little or no blood in the urine samples. The only glow was seen on the plate with bovine blood (Table 1).

With the UV test, only the Doe In Estrus® lure yielded a 3, although all other samples glowed, yielding a 1 or 2. Water did not glow and was assigned 0. These differences in intensity could again be correlated to how diluted each type of urine may be (Table 1).

The 100% cotton t-shirt UV test yielded all negatives. However, in the UV test for untreated cotton, the following intensities were recorded for each urine-based lure: Code Blue® Scrape Mate, Active-Scrape®, Code Blue® Buck, and Trail's End® #307 were given a 2 and Doe In Estrus® was given a 1. The rest (Code Blue® Doe, Code Red® Buck, and Primetime® Natural Doe) had no glow (Table 1). The same results were reported after 24, 48 and 72 hours.

For the leather boot UV test, when the samples were still wet, some of the urine that had not soaked in created a glow. But when all the urine dried, no glow could be seen on the leather for any of the urine types (Table 1). Some of the urine had dripped down onto the foam rubber sole of the boots and a peach-colored glow could be seen. Thus, we believe many other types of fabrics (solid rubber, polyester canvas, denim, etc.) could be tested in the future.

In the field UV test, some glow could be seen right away in Trail's End® #307, Code Blue® Scrape Mate, and Active-Scrape®, Doe In Estrus®, and Code Blue® Buck tests. However, most of the urine-based lures immediately soaked into the ground and could only be picked up on leaves and sticks on the ground. After 24 hours, the site was re-visited with the UV light, but only tiny flecks of dried urine could be seen on solid surfaces. These tiny flecks glowed peach and were assigned a 1. Trail's End® #307 glowed the most after 24 hours and was assigned a 2 (Table 1).

Our tentative results suggest that each test has limitations in regards to detecting urine-based lures. The most promising results were provided by the UV tests. However, we found that urine-based lures could not be picked up in certain fabrics like leather and treated cotton shirts, but could be picked up on solid ground surfaces (i.e., leaves, sticks), untreated cotton and shoe foam.