Peck Farm - Phase 2.5 Report

Phase 2.5 of the Peck Farm research will concentrate on the findings of Orange 1 and Yellow 1 that were found to be rectal positive in April 2018 after their respective 3rd biopsy.

It would be beneficial to the reader in fully understanding this report and the deer's history to review both the Phase 1 and Phase 2 reports located at (http://www.whitetailsofwisconsin.com/wcff.php). This will provide background information pertaining to the historical preclinical to clinical status of the 2 deer in this current report and the other deer on this farm. Future information will be presented in the Phase 3 portion of the study.

To provide a more in-depth comprehensive report for CWD positive status deer, collected samples from deer must have an approved USDA Federal Permit for the possession, transportation and testing of positive CWD materials. AOS has developed this working relationship with the collaboration of a Federally approved facility for this effort to continue and provide these in-depth investigations of CWD positive status deer. Funding for Phase 2.5 was provided by Whitetails of Wisconsin Cervid Farmers Foundation, North American Deer and Elk Farmers Association and Deer Breeders Corp. and AOS for this review.

Orange 1 was found dead on September 5th and Yellow 1 was found dead on December 26th, 2018. In this study, pre-scheduled gross necropsies were to be performed on any deer that dies from any cause including spontaneous death. We were able to collect certain samples that provided information to support the industry's research objectives and interests in why a deer develops illness and later dies with or without detectable CWD.

Background

In referencing the information already provided, both deer in this report (Orange 1 / Yellow 1) were rectally tested in the spring and again in the fall of 2016. At this time both, animals were determined to be rectal negative by IHC methods for the CWD prion. Upon their 3rd rectal testing in April of 2018 the results for both deer indicated that they were rectally positive for the CWD prion via IHC testing by the NVSL.

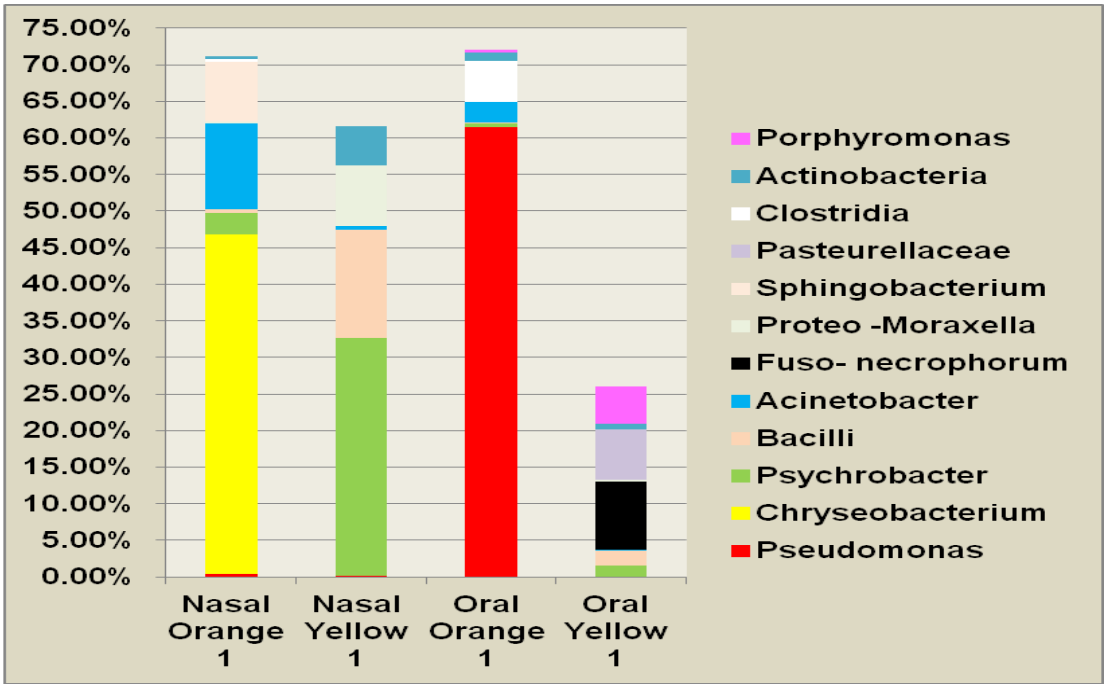
Yellow 1 Left, Orange 1 Right Spring 2018 detected CWD+ by 3rd rectal biopsy.

Typically, by using the rectal biopsy method in a herd to detect at least 1 positive deer with confidence, the average herd size of approximately 100 deer would be needed to provide a sensitivity rate of approximately 76% for the GG and 42% GS genotype respectively by IHC methods (Nichols et al).

These percentage rates are described to be specific to each group of deer with the same genotype. If there is a mix of genotypes within the herd, the percentage rate of a positive detection could be diminished for detection.

With Orange 1 and Yellow 1 detected to be rectally positive in April of 2018 (avg. 59% sensitivity) and negative in April of 2017, it would place both of these deer in an estimated unknown stage of clinical infection. In a predictive sense, if we took the average number days from official detection via rectal biopsy, until their death time of onset would predict fall 2017. This is using an average of 6 months prior to detection. This is an interesting point since these time frames (Aug-Nov.) are transition timeframes hormonally in deer as they typically put on their winter coats and underlying body fat.

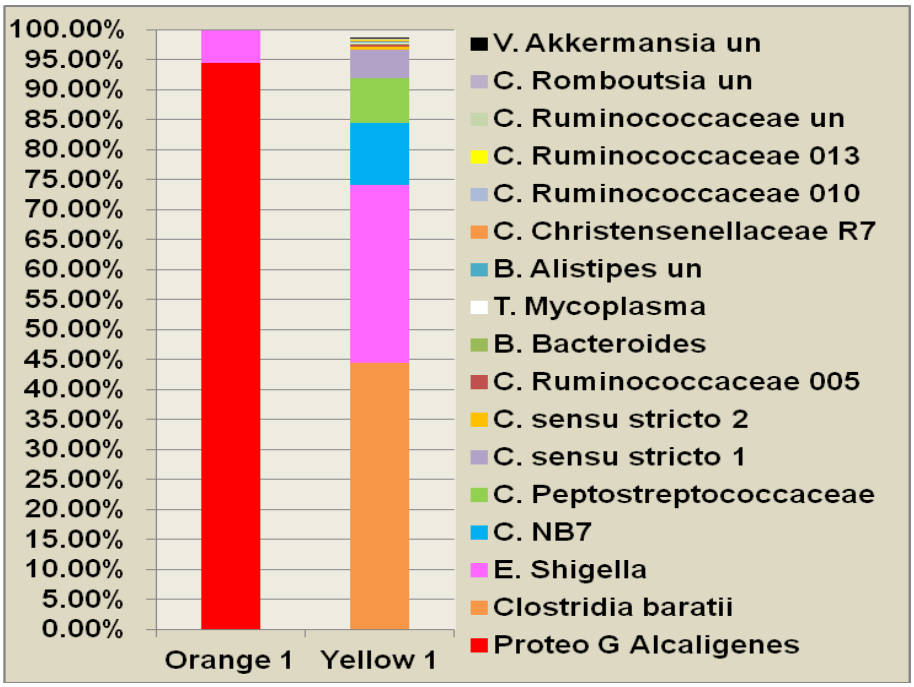
Samples for bacterial analysis were collected for nasal and oral swabs on both deer (Table 1).



(Table 1) Nasal and Oral samples from Orange 1 and Yellow 1. Both deer differed greatly in both sample organisms though negative organisms predominated in both deer.

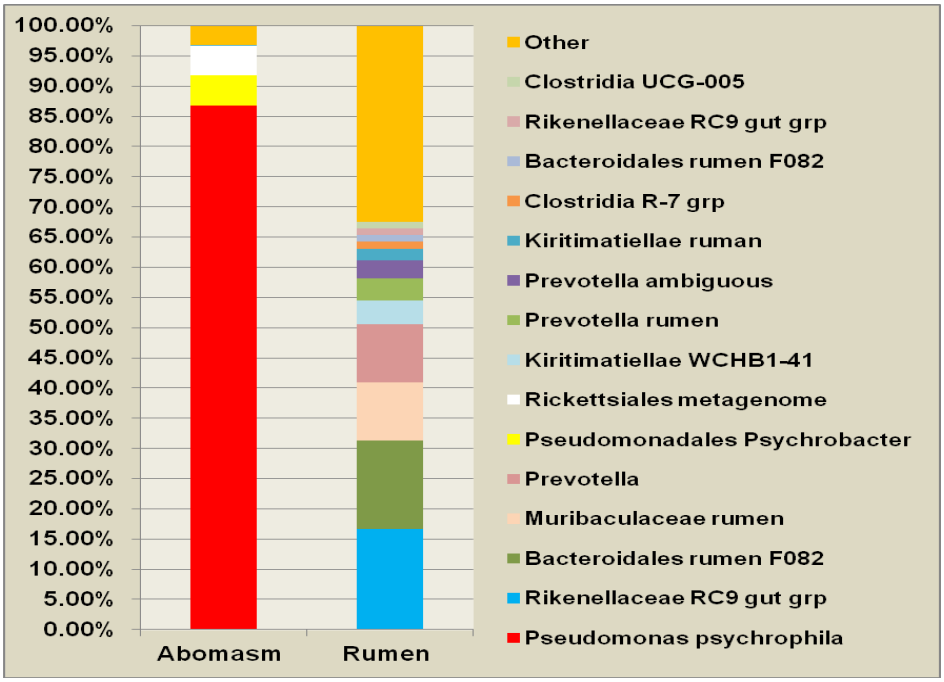
Blood samples were also taken (Table 2). Three negative organisms (Proteobacterium Alcaligenes, Clostridia baratti and ecoli Shigella) were present at a high level and could have contributed to diminished health of these 2 deer. These organisms will be monitored for presence

in the other deer on this quarantined farm in addition to the 2 control farms in this study. To date, y these organisms have not been found in deer on any other farm.

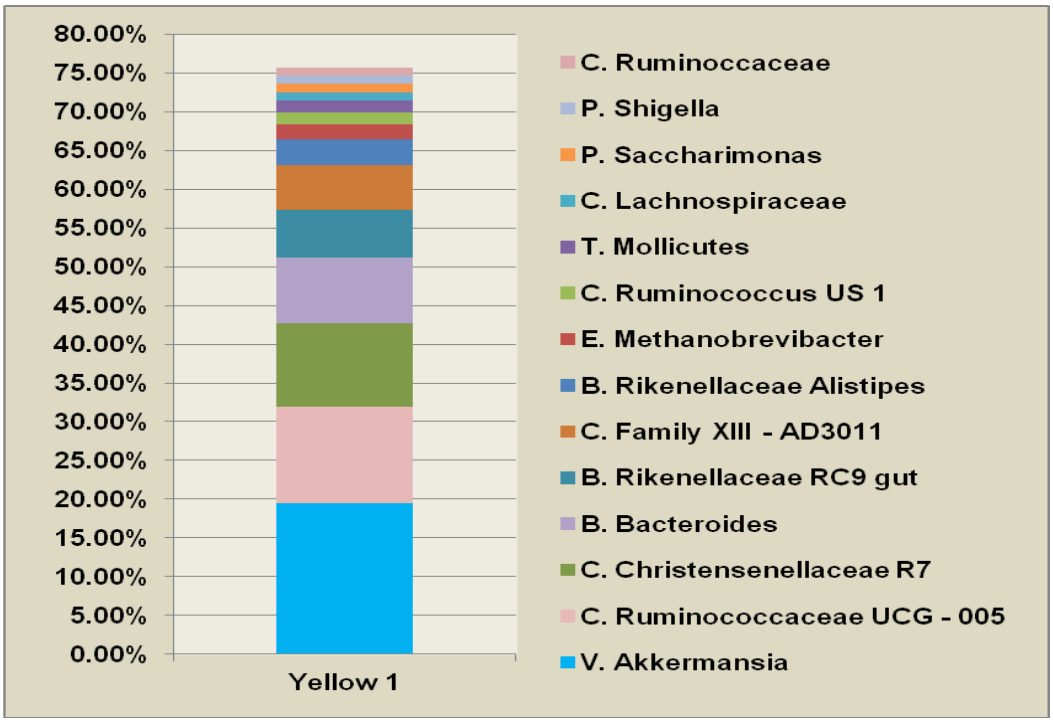


(Table 2.) Blood samples demonstrate a negative bacterial presence in both deer's blood. Though distinctly different, these organisms may represent 2 different states of clinical infection.

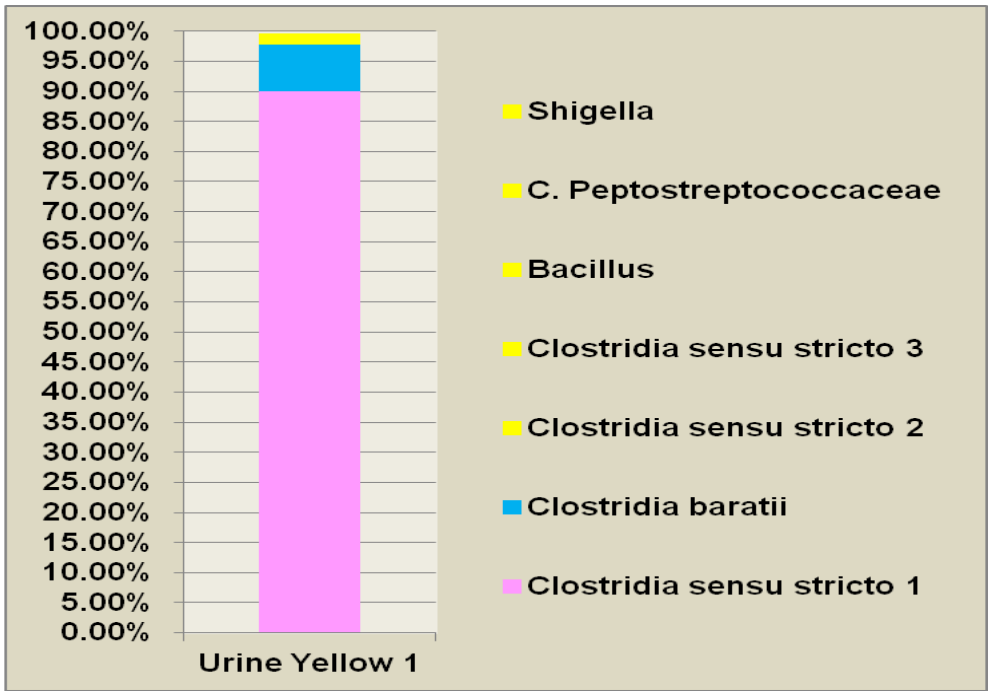
The below data represents samples collected from abomasum and rumen of Yellow 1. Samples were not collected on Orange1 earlier because an allowance permit for collecting and shipping positive CWD samples had not yet been obtained.



(Table 3.) Yellow 1 associated negative bacterial loads upon death



(Table 4.) Yellow 1 fecal analysis demonstrating Akkermansia as the predominant organism. This organism helps to maintain the intestinal mucosal integrity.



(Table 5.) Clostridia species were dominant in the urine of Yellow 1.
C. baratti was also documented as the highest organism present in this deer's blood.

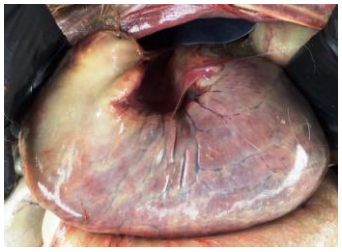
The following details describe the necropsy findings of Orange 1 first and then Yellow 1 in an abridged manner. Full necropsy reports can be reviewed online @ (http://www.whitetailsofwisconsin.com/wcff.php).

Orange 1

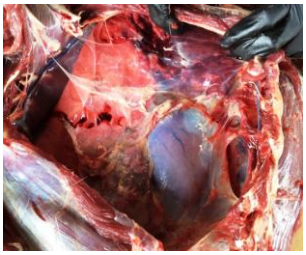
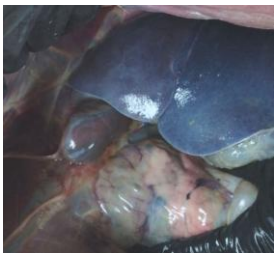
Dr. Amy Robison (herd veterinarian) was notified by the herd owner on September 5th 2018 that the deer was found dead.
The pre-determined plan of this study was to perform any gross necropsy on the farm vs. transporting the deer to the veterinary clinic to protect from cross contamination of facilities or equipment during the necropsy or tissue harvest.

Orange 1, born 6-7-10, was determined to be 8.16 years was found dead on 9-5-18 with a genotype 96 GG.

Dr. Robinson's clinical observations upon opening the abdominal cavity: this deer looked to have poor digestive absorption due to a malformed (A.) abomasums, (B.) impacted pylorus, (C.) pyloric stricture (D.) water filled rumen with (E) sand.

A. 
B. 
C. 
D. 
E. 

Upon further review of the internal organ systems it was also noted that Orange 1 had (E) cranial ventral Pneumonia, (F) lack of fat of both kidneys as well as (G.) omentun.

E.  F.  G. 

With an internal organ system being compromised from the initial restriction of feed passage the proper function of the abomasum would be key to providing a normal digestive process supporting proper nutrition of the deer. The reduced nutritional status was further compromised by an observed parasite load. Higher parasitic loads in deer (coccidian, stronglyles) can lead to further nutritional stress and a compromised immune response. This type of nutritional stress could make the deer more susceptible to additional diseases originating from common environmental contaminants.

Fecal assessment in the early fall of 2017 revealed stomach worms. The whole group of deer in this study was subsequently wormed 2x using a commercially approved ruminant wormer in a pelleted feed mix in the fall 2017.

In April 2018, upon sedation for the rectal biopsies, fecal collected was performed for each deer in the group and assessed for parasite load. The results showed each deer to be negative for any parasites. Since there was no naturally growing forages in the deer's pen no further worming was to be anticipated. Other than supplemented dried hay provided to the deer for the lack of forages it was thought that a low, to no, prevalence from natural parasitic involved grazing would be of concern.

In further review of parasitic information, Strongyles generally as adult parasites, can lay several thousand eggs each day before completing their life cycle. The parasites entire life cycle can take six to seven months. There are other large strongyles (Strongylus endentatus and Strongylus equinus) that have similar life cycles, but their larval migration is primarily through the liver.

Some common stomach worms in the environment that are of concern with cattle are *Haemonchus placei* (barber's pole worm, large stomach worm, wire worm), *Ostertagia ostertagi* (medium or brown stomach worm), and *Trichostrongylus axei* (small stomach worm). Stomach worms have two presentations of Ostertagiasis and of parasitic gastro-enteritis in cattle.

These parasitic presentations are generally noted as:

Type 1: Adult worms living in stomach attached to the lining, causing damage to the gut lining and deprive host of nutrients. Usually occurs in grazing season. Animals affected usually don't thrive well and may have diarrhea.

Type 2: Larval stage 4 burrows into stomach lining and emerge in large numbers causing explosive diarrhea and may even be fatal. Usually occurs in December/January in the northern hemisphere following cold weather hibernation in the autumn but can occur in the autumn following warm weather hibernation in the summer.

Of interest it was noted that a moderate amount of coccidian organisms were present in Orange 1. The source of this parasitic contamination could be spread into the deer's housing area on this farm by access by other wildlife to the equipment such as feeders and water supply. Other wildlife can defecate / urinate onto the deer's feed or drinking water increasing the opportunity to cross contaminate negative organisms to your deer.

Other samples collected post death in Orange 1 included liver to assess mineral status. On the pathologist report, a high selenium level of 2.0 ppm was noted. The question then was raised concerning over supplementation of selenium as a possible source. Since we had already reviewed the mineral components of the water source in Phase 1, water was discounted as a potential source. A feed sample was submitted for a heavy metals review and was found (1.02 ppm) to be within the parameters of the liver panels range (0.20 – 1.10ppm) parameters.
Though it was reported as selenium being high, in the lab testing report, it was determined that feed and or water sources were not a source of this nutrient imbalance. A plausible reason of any identified nutrient imbalance could be cascading negative health related to the deer's inability to properly utilize nutrients. This is reflected in the observations from Dr. Robinson and comments on the constriction of the abomasums which could affect the entire digestive tract.

Yellow 1

Yellow 1 was an unexpected death in the study. Though the Does winter hair coat came in light, she was still active, ate apples and acting normal like the other deer. Maybe it was obvious that the other deer in the pen thought different. Getting a call the day after Christmas of her death was a reality check that no day is a good day when a deer dies unexpectedly. Dr. Amy Robison (herd veterinarian) was notified by the herd owner on December 26th 2018 that this deer was found dead to arrange for sample collections.

Yellow 1 – noted as doing well 7 days before her death

Any animal death expected or not, provides us the opportunity to use our testing methods to understand progression of the disease process we have come to know only as CWD. In the post mortem of Yellow 1 it became obvious that the other deer were picking on her in her last moments (H.) Though her rumen was considered normal in appearance (I.) her abomasm had some developing ulcers (J.) Her kidneys (K.) appeared normal but lacked the fat content like her abdominal cavity (L.) showed. She was determined not to be pregnant due to her uterus (M.) being empty.

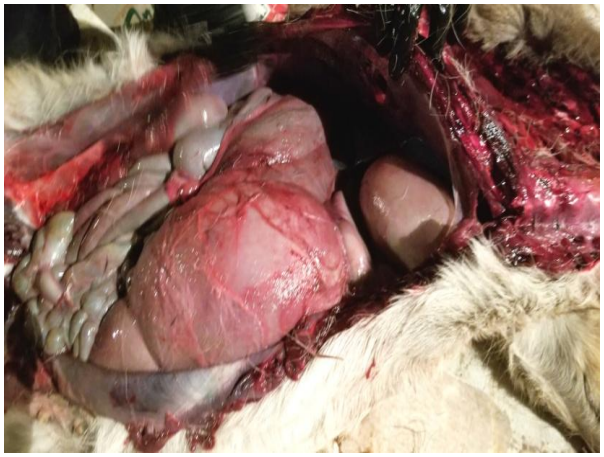
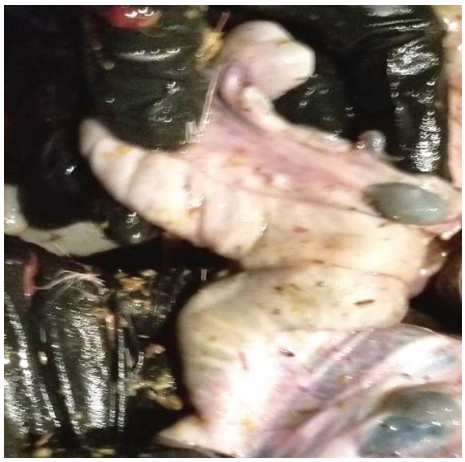
In respects to the findings of both deceased deer it is always important to collect specimens for finding a cause of death. Though sometimes not practical or financially feasible these deer provided an important insight to the study of a disease process.

H.  I. 

Yellow 1 - severe bruising (H.) under hair coat the rumen (I) appeared to be normal

J.  K. 

Yellow 1 abomasm (J.) developing gastric ulcerations, (K) kidneys appeared normal

L.  M. 

Yellow 1. Abdominal cavity (L.) lacks body fat stores, (M.) Uterus noting no pregnancy

Discussion

In summary, the follow up to the health end points of Orange 1 and Yellow 1 in this report will add to the information we have learned to date. In following these deer biological profile and reviewing their acquisition of multiple negative disease organisms contributes to a disease process leading towards a deer dying with detectable CWD. The disease called CWD is an end life stage disease found typically upon death with an unknown time of indexed exposure to manifest itself to a clinical case. The process of this review continues to show us through appropriate surveillance testing, that early detection of common negative health-associated environmental organisms is critical to the deer's health and wellbeing. The geographic location of environmental organisms, water or feed could represent an opportunity to exposures to negative associated organisms which could unknowingly start the cascade of diminished health. This would lead to increased risk potential for your deer to manifest towards a more critically oriented disease process over time. Orange 1 and Yellow 1 had these base negative organisms on board (mycoplasma / ecoli shigella) for at least 2 years prior to converting rectal positive upon their 3 rectal exam.

Continuing into Phase 3 will provide more detailed insights into the health of the rest of the deer on this quarantined farm. By continuing the health monitoring of the remaining 4 deer (Does - Pink 1- 96gg, Purple 1- 96gs, Yellow 2- 96gg and Buck Red1- 96gs) in review of their 4th rectal biopsy and continuing medical health surveillance will continue to provide you the farmer insights into building your bio security and beat management plans on your farm.

Your Bio security plan should include your relationship with your veterinarian for health surveillance and maintenance of your deer. In reviewing your farm practices, you should always error on the side of caution when supplying clean water, feed, hay or control of vermin (crawling/flying) that has the ability to come onto your farm. If you do not follow up on requiring testing of your hay or feeds for nutritional quality for unwanted contamination of high mineral or pathogen contamination puts your deer at unnecessary risks.

Additional information not covered in this report (fungal) will be presented in the Phase 3 report as a continuance in providing a health comparison of the remaining deer in the study.
Both necropsy reports will be submitted with this report for membership review online.

Special thanks goes out to Dr. Amy Robinson of Military Ridge Veterinary Clinic for her continued Cervid research efforts and to you the members of Whitetails of Wisconsin's' Cervid Farmers Foundation (WOW / WCFF), North American Deer Farmers Association (NADeFA) , Deer Breeders Corporation (DBC) and Agricultural Omega Solutions LLC (AOS) for continued financial research support.

Submitted: Jerome Donohoe

Disclosure Statement: Though there are Non - disclosure agreements with the farms in this study to protect confidentiality and any perceived research Bias , A.O.S. declares there are no conflicts of interest generated with or between WOW / WCFF Foundation, NADeFA or DBC as designed and funded.