Peck Farm Research Report

Phase 2

Title: Identifying Nutritional Characteristics' of a Cervid Farm under CWD Quarantine

By: Jerome Donohoe
Scientific / Educational Committee
Wisconsin Cervid Farmers Foundation

Hypothesis: A Cervid supported with optimized feed, forage and water along with its genetics can stave off an initial or continuing disease process that could lead to the onset of a disease process like a neuro-degenerative disease called Chronic Wasting Disease (CWD).

Whitetails of Wisconsin's' Cervid Farmers Foundation (WCFF), North American Deer Farmers Association (NADeFA) and Deer Breeders Corporation (DBC) have continued to collectively fund this research investigation into the understanding of an initial disease process' based on nutrition or lack of nutrition. This continuing investigation will help the members of the Captive Cervid Associations as well as other Wildlife Agencies in the understanding compounding disease processes or nutritional processes that could lead to a progressive neurodegenerative onset and subsequent mortality of cervids involving a detectable Chronic Wasting Disease. Our test farm continues to exist under quarantine conditions in a CWD endemic area as the wild deer population continues to increase in CWD detectable contamination in post mortem deer.

Phase 1 Background

The current test farm had been considered CWD negative since 2002 until one deer (1.5 year male) died from a goring incident in early January 2016 from another male deer (10 year old) in the same pen. Upon submitting the deceased deer's tissues for CWD testing it was noted that the deer tested positive for a progressive neurodegenerative disease (CWD). Testing results by IHC of submitted samples showed positive detection in tissues of lymph nodes and the brain. This was the first detection of CWD for this farm since required testing began in 2002.

Since this farm is a single fenced farm, the CWD is speculated to have come from the wild deer source in the surrounding area for which is a high risk endemic area of the State (Iowa County) for CWD positive wild deer. Another possibility was a spontaneous disease event resulting from a neurodegenerative process which has lead to this research investigation.

Upon detection of this neurodegenerative disease, a request was offered by the farm owner to the Whitetails of Wisconsin organization for research review to find out why this disease happens. This request was assigned to the Wisconsin Cervid Farmers Foundation to conduct scientific research in a Phase 1 proposal as an effort to study the deer on this farm for primary nutritional assessment and subsequent disease process leading to the onset of a later in life neurodegenerative disease called Chronic Wasting Disease (CWD). See Phase 1 report www.whitetailsofwisconsin.com

Phase 1 Summary Review

A phase one research review of a whitetail deer farm, held under quarantine for CWD, was to help answer the hypothesis: A Cervid supported with optimized feed, forage and water along with its genetics can stave off an initial or continuing disease process that could lead to the onset of a disease process like a neuro-degenerative disease called Chronic Wasting Disease (CWD).

The endemic area where the quarantined farm is located generally has a prevalence rate above 15%* for wild deer which routinely post mortem test positive for CWD in the population annually.

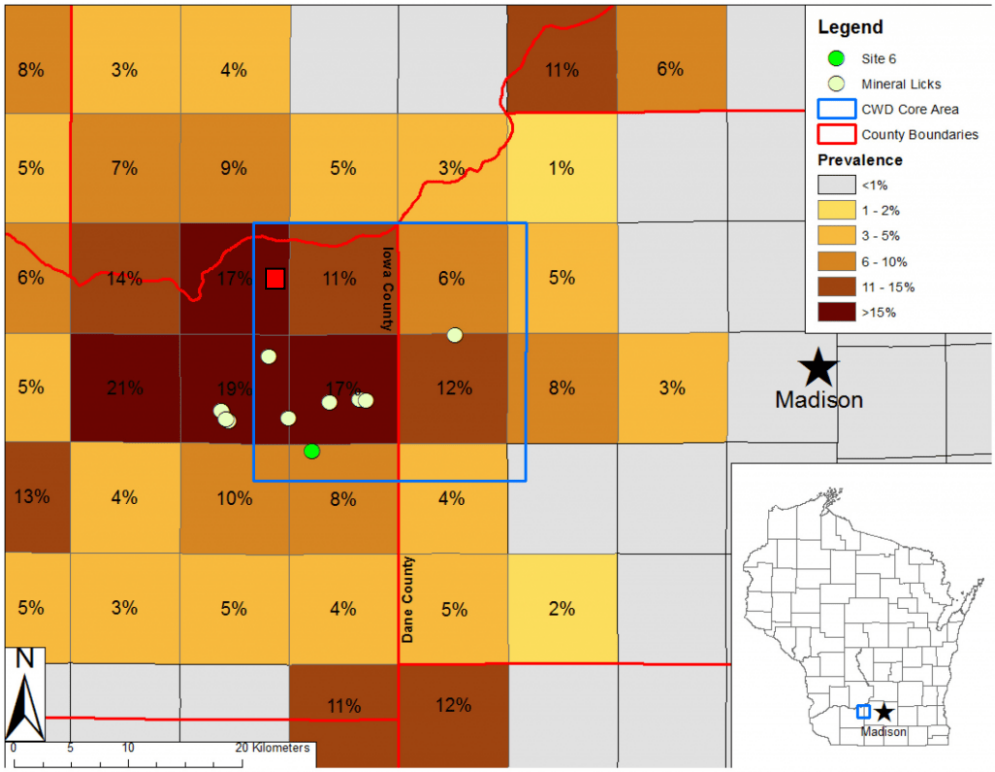
The initial review of our farmed deer showed an improved body condition through a positive nutritional intervention during the first year of re-conditioning. Though improvements were made, nutritional deficiencies left this small herd (6) without any pregnancies for the spring of 2017.

Other areas of interest resulting from this first review include deer farmers paying close attention to on and off farm supports for their deer regarding water quality, feedstuffs, animal transfers and sanitation practices by embracing a developing Bio-Security program.

Review of the nutritional and bacterial status of the deer on the quarantined farm and the 2 control farms provided a basis of pre-clinical data for future follow up on these farms in the next Phase of the research. It was determined that a continued ongoing review of the Phase 1 components is warranted as the onset of clinical signs of CWD could take multiple years.

*** Mineral licks as environmental reservoirs of chronic wasting disease prions**

Read the full story here: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0196745#ack



The importance of having a key Deer Farm location for Research to study developing disease transmission near trace mineral licks. Whitetails of Wisconsin / Cervid Farmers Foundation, North America Deer Farmers Association and Deer Breeders Corp. team up to conduct research on a deer farm quarantined for CWD exposure.

Phase 2 Research rational:

To maintain the continuity of the pre-clinical data produced from the Phase 1 study, the deer on the farm currently under CWD quarantine and the same 2 control farms with a continued health certificate of CWD negative status' will be used.

These farms continue to be maintained on a NON-Disclosure basis as to maintain their privacy and to remove any research bias of the study.

My research approach for Phase 2 is based off of Phase 1 Industry funded results for which were supported with supplemental pre-clinical research funding by Agricultural Omega Solutions LLC. (AOS). Phase 1 results covered sample collections and determinations to include water, feed, fecal, parisitology of the deer on the 3 farms. Subsequent funded research findings were provided by AOS was for forages, saliva, probiotics, blood, urine and tissue samples (lung, liver and brain). These base and additional samples are helpful in determination of future proposed research for best materials for collection in refining the process. This helps in expediting areas of concern for pre-clinical sampling prior to early onset of disease detection and or identification.

In supplemental findings, Phase 1 demonstrated that there are certain gram negative bacteria (clostridia, mycoplasma, etc.) detected in blood sampled. Further, these gram negative disease causing organisms were found in the water, feed or other environmental exposures on all 3 farms as well as initial samples collected from a wild deer living in close proximity of the quarantined farm. These findings led to a direct link to bacterial infection within the blood of the 2 deceased deer (Green 1 Doe / Blue 1 Buck) as well as the brain of the buck. The rest of the deer on the quarantined farm also showed various levels of the same disease causing organisms in the collected blood. Upon the Buck (Blue 1) and Doe's (Green 1) death, lymph nodes and brain tissue were submitted for IHC testing for CWD detection by the USDA in Ames Iowa and was subsequently found to be certified negative for any neurodegenerative disease process called CWD.

These preclinical findings demonstrate that an immune system, compromised by an overload of gram negative bacteria could lead to a negative health consequence. Without this type of a surveillance plan on the farm an apparently healthy deer herd could be at considerable risk for a negative health status as a result of nutritional deficiencies. These deficiencies can lead to additional cascading disease processes which impact breeding success, developing an immunological disease or a neurodegenerative disease process called CWD.

Gram negative bacteria can alter gene expression leading to multiple organ dysfunction of the deer over time that leads to a reduced immune status and the ability of the deer to ward off acquiring other disease process' or the potential of developing a neurodegenerative disease such as CWD.

By generating this new information, as compared to past and ongoing research by AOS, potentially unwanted disease causing organisms of the whitetail deer (saliva, rumen, and lung, liver, urine, fecal, blood and brain) can now be reviewed for comparative animal health status.

Refinements in the Phase 2 study design, for collecting samples for testing, will look to show a direct correlation and understanding of where negative bacteria reside in the deer's blood and feces. This

will provide new information for determining vaccine status, parasitic status, as well as overall deer health providing general information about a deer's immune status. Excessive numbers of gram negative bacteria found in the blood have also been found in the brain and are associated with a debilitated immune system leading to a negative health outcome.

PHASE 2 – Methods and Materials

Since the first deer (on the test farm) was detected to be positive upon its death (Jan. 2016), it has been about 1.5 year. Two sets of rectal biopsies (May and Aug. 2016) were performed on the deer with negative test results.

Another round of rectal biopsies is planned (April 2018) to update the status of the deer on the quarantined farm in determining rectal biopsy status potential of an early onset of the neurodegenerative disease process (CWD).

Hair samples will also be collected for DNA analysis of the deer to review their genetic resistance status utilizing new markers of resistance. These markers for gene resistance to the early onset of a neurodegenerative disease process could hold promise to the future of deer farming.

Saliva samples will be collected on each deer for comparison to other negative associated bacteria associated with dietary inputs. Blood samples will be taken to compare the bacterial load with the original bloods retrieved upon sampling all deer in May of 2016. Two fecal samples will be taken for parasitic load and for bacterial screening.

METHODS

In order to initiate any action plan for the collection of samples it is necessary to form a team with an action plan to achieve the objective. Members from the Whitetails of Wisconsin volunteered and were on hand to initiate a darting protocol commonly used in the captive cervid industry.

Supplies used for this study included rectal tissue cassettes, paper envelopes for hair, sterile swabs for saliva, EDTA blood tubes, test tubes for fecal collection, 1 Bam Kit (1 bottle BAM with reversal) and 6 doses of Excede, a supportive 3-5 day antibiotic for the deer post reversal.

WOW/WCFF members in PPE from left to right: Joel Espe, Herd Veterinarian Dr. Amy Robinson, Brad Heath, Ray Hanson, Jerome Donohoe and Rick Vojtek. Thank you for helping.

The darts used for this procedure were donated (Pneu-Dart) as well as the anethstetic “Bam’ (ZOO Pharm). The anesthetic was supplied through a prescription through the farms herd veterinarian. With challenges at hand and the deer’s co-operation, approximately 20 -30 minutes all of the deer were sedated to a level appropriate to begin our multiple sample collections.

Each deer was subsequently reviewed for general overall health profile before sampling. This includes their overall resting position and respiration rate since all deer react differently when anethtisized. A protective cloth covering was placed over each deer’s face area as to cover their eyes from any dirt or debris and to keep them calm while antehtisized. As each deer was sampled verification of ear tag numbers, hooves and other general health / body conditions were also reviewed. Only one deer needed to have their back hooves trimmed as being considered too long. After each deer was sampled, they were provided a reversal drug (BAM Kit) for the anesthetic along with an antibiotic which is a general practice for anesthetized livestock while under veterinary care. All samples were collected by or under the direction of the herd veterinarian. Each deer was visually monitored as they came out of anesthesia to prevent unanticipated outcomes. All deer recovered very well and now on to testing the samples.

Quarantined deer in study Left to Right: Buck Red1 and Does Yellow 2, Purple 1, Orange1, Yellow 1 and Pink 1.

With all samples collected there is a sense of anticipation for updating our herd’s health status of awaiting a past elusive positive CWD rectal test result. This sounds strange in wanting CWD but to want CWD in a live animal model is a unique opportunity and a must for one to collect pre-clinical samples to understand the initial deer’s health status. This is important to the study for long term survivability of the deer on this farm, your farm and in wildlife.

Control farms described in Phase 1 collected their respective blood and fecal samples for support of this Phase 2 effort for maintaining research continuity for control conditions on their farms and paid for their own collection expenses (materials, labor) including shipping of samples.

Since pre-clinical samples were obtained from these deer prior to Phase 1 it is important to have a continued sampling of the deer for updating health assessments. These follow up assessments would be to optimize our knowledge of the deer health status and or any noted disease progression. This health monitoring along with other field observations will result in developing a reliable standard of monitoring health and disease processes in the whitetail deer that seems to be lacking today for the cervid industry and wildlife agencies alike.

Results

Rectal Biopsies

The deer sampled for CWD testing are noted (Table 1) as to their respective results (N= negative, + = positive) for rectal biopsy, lymph node or obex by IHC in the table. All other deer tested negative status by IHC for rectal biopsies. With 2 rectal positive deer (Yellow 1+ and Orange 1+) now on board we can now monitor the disease progression as compared to past control data and other current samples collected for a more clear disease progression. Throughout this report positive deer will only be noted as Orange 1+ or Yellow 1+ for chart data description.

Deer ID Tag	DOB	Genotype		Deer ID Tag	DOB / DOD	Genotype	
Doe Orange 1	6/7/2010	96GG	+	Buck Unknown	death - 1/9/2016	n/t	+
Doe Pink 1	5/20/2012	96GG	N	Buck Blue 1	5/1/2006 - 8-1-16	96GS	N
Doe Yellow 1	6/4/2012	96GS	+	Doe Green 1	6/4/2015 - 8-1-16	96GG	N
Doe Purple 1	7/8/2012	96GS	N	Orange 2 (fawn)	6/1/2016 - 8-1-16	n/t	n/t
Buck Red 1	6/4/2015	96GS	N	Control Farm B		96GS	N
Doe Yellow 2	5/20/2016	96GG	N	Control Farm B		96GS	N

Table 1. Left column - Deer listed by ID, Age, Genotype and CWD status. Right column – Deceased deer from farm by ID, Age, Genotype and CWD status. Control Farm B deer by Age, Genotype and CWD status.
(N = Negative, + = Positive)

Yellow 1+ (left) and Orange 1+ (right) body condition profiles on July 3rd (center) and July 16th.

DNA Hair Samples

In historical studies, there is a scale of resistance among WTD genetically typing as 96GG, 96GS, and 96SS. Among WTD with variation at PrP 96, 96GG WTD are most susceptible to the onset of CWD while 96SS WTD are somewhat resistant to CWD with 96GS WTD are intermediate between the two. Among the WTD with variation at PrP 226, 226K/96G WTD are considered rare, and may be more resistant as the 96SS WTD to CWD, while 226KK animals are extremely rare and likely highly resistant to CWD. 96GS/226QK animals are also quite rare, and would be expected to be even more resistant than 226K/96G animals since they have both a 96S allele and a 226K allele.

A similar story may be the case with 95H/96G animals – they are more resistant than 96GS animals, though not quite as resistant as 226K/96G animals. 95HH animals are very rare, though are likely to be more resistant than 96SS animals, and not quite as resistant as 226KK animals. 95H/96S animals are also rare, though are likely to be more resistant than 96SS animals.
In relation to the deer in this study submitted hair samples for DNA testing did not see any variation in the 226 or 95 loci, though 4 deer genotyped as 96GS.
One finding from the hair testing was the prior unknown genetics of Yellow 2 since the deer was not tested prior as a fawn. Though both parents (Blue 1 / Yellow 1+) were of the GS genotype, Yellow 2 typed out as a 96GG. (Table 2) This isn't unheard of as in all genetics there'll be a 25% chance of that happening. 50% of those fawns would be 96GS, and the other 25% would be 96SS. (Table 2.)

Parent 1	Parent 2	Offspring		
96GG	96GG	100% 96GG		
96GS	96GG	50% 96GG	50% 96GS	
96GS	96GS	25% 96GG	50% 96GS	25% 96SS
96GS	96SS		50% 96GS	50% 96SS
96SS	96SS			100% 96SS

Table 2. Breeding for genotype percentages showed a regression from both parents in our herd being GS / GS that produced a GG typed offspring.

Review of deer by ultrasound showed Pink 1 to be pregnant!
Born July 20th the buck fawn did not make it to the following day.

Saliva

Providing a constant feed and water source tends to provide for a more stable bacterial organism base in the mouth of the deer. Seasonal changes from winter to spring, summer and fall provides a seasonal variation to these base bacterial organisms affecting the makeup or concentration of the bacteria in the mouth. Seasonal changes that occur from spring to winter provide the most challenging time of the deer's exposure to environmental bacteria. Environmental bacteria flourish while your deer are switching from traditional feedstuffs to green forages that change the nutritional intakes from the winter months. Such a change from feed, dry hay, trace minerals, supplements or treats will alter the base bacterial load in the mouth either to a good, balanced or bad bacterium. This could also happen as deer are moved from pen to pen or farm to farm whether instate or out of state when moving, buying or selling deer.

In Phase 1 feed and water supplies were unchanged and were maintained on the farm to monitor the dietary intakes. This was necessary so as not to make changes that could potentially provide misleading or confounding nutritional status, if we by chance had a change in our CWD status from negative to positive.

Forages tested in Phase 1 identified either positive nutritional components (clean hay) or multiple negative bacterial organisms or trace mineral contamination (dirty hay) that could build a negative nutritional profile for your deer.

Now that we know we have a CWD status change (Orange 1+ / Yellow 1+) we can now compare what organisms reside in the mouth of the deer via saliva (anti-mortem) from positive or negative exposed deer. It is assumed that all deer on the farm have the same bacterial profile as they are eating the same feed or drinking from the same water source so we believe that all of our deer must have the same bacteria. Deer like us and other animals are unique individuals. Each one has a unique reservoir of bacteria for aiding in digestion of the foods we eat for survival. Daily feed and water intake can harbor different good or bad bacterial organisms depending on their sources (water, hay, feed, supplements) including from different geographical origins. A change in dietary intake (deer movement) provides any deer with a positive or negative dietary challenge that can have an impact on their saliva bacterial makeup or concentration.

A variety of feedstuffs commonly offered to deer were tested for bacteria loads to determine what organisms in feedstuff could resonate in the deer's saliva, blood or fecal samples on the quarantined farm as well as control farms. (Table 3 – 7 - Percentages† are by volume).

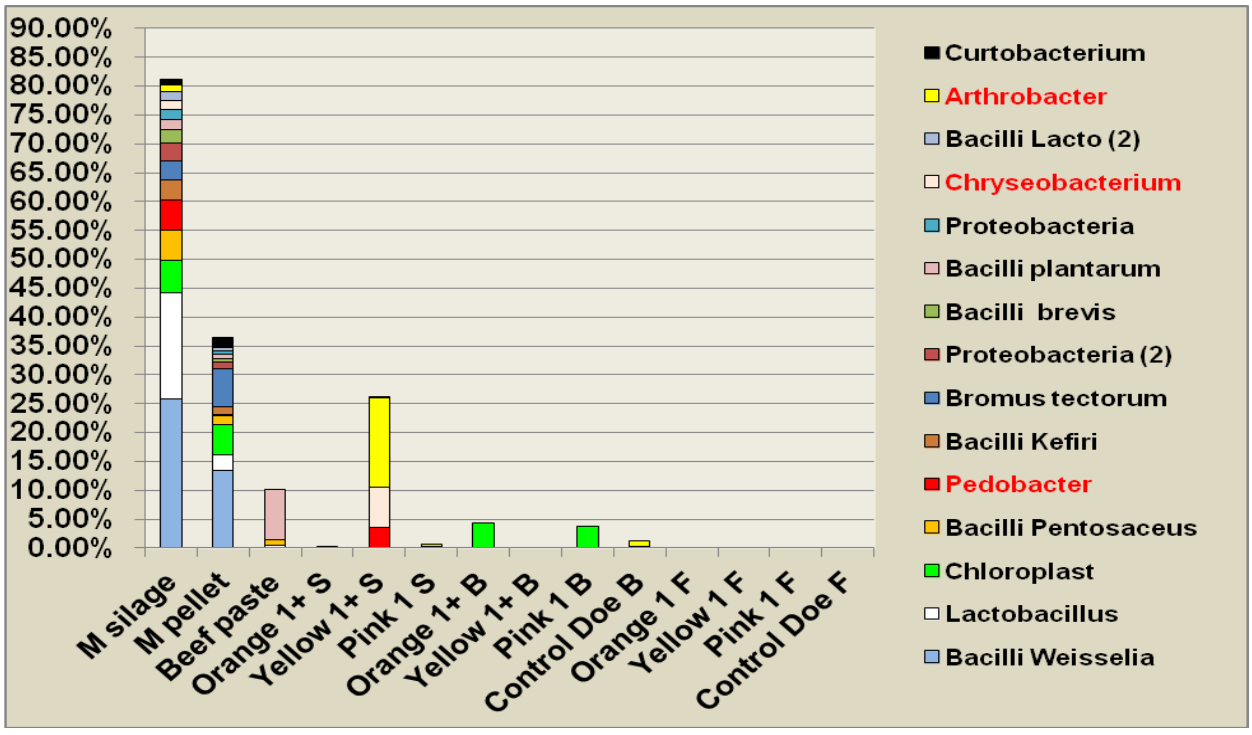


Table 3. Bacterial organisms present (17) from 25% down to 1% in volume for silage, silage pellets, or beef paste suppliment. Bacterial organisms were matched from the silage or silage pellet to the deers saliva (S),blood (B) or fecal (F) of whitetail deer. Only 3 negative bacterial organisms listed in red show growth from silage in the deer Yellow 1+ saliva but not blood or fecal.

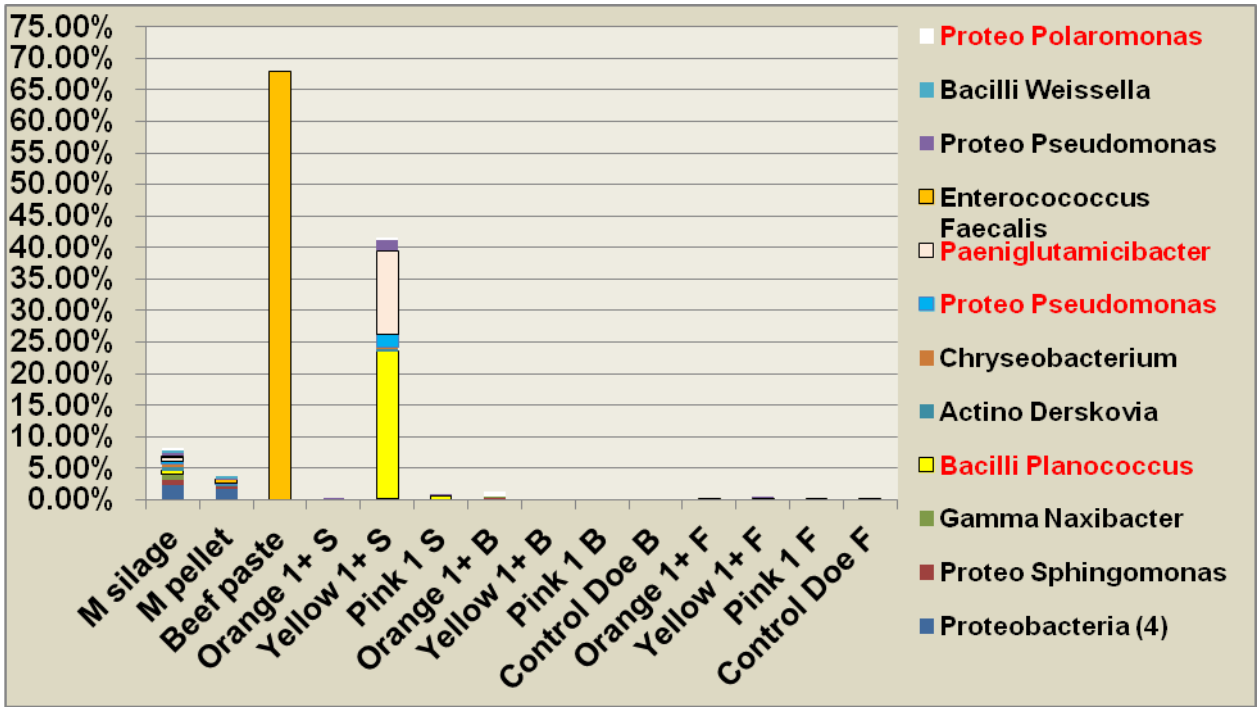


Table 4. Bacterial organisms (15) were identified (0.8% down to 0.4%†) in silage that showed 4 organism related to the saliva of Yellow 1+ but not in any other deer in the study.

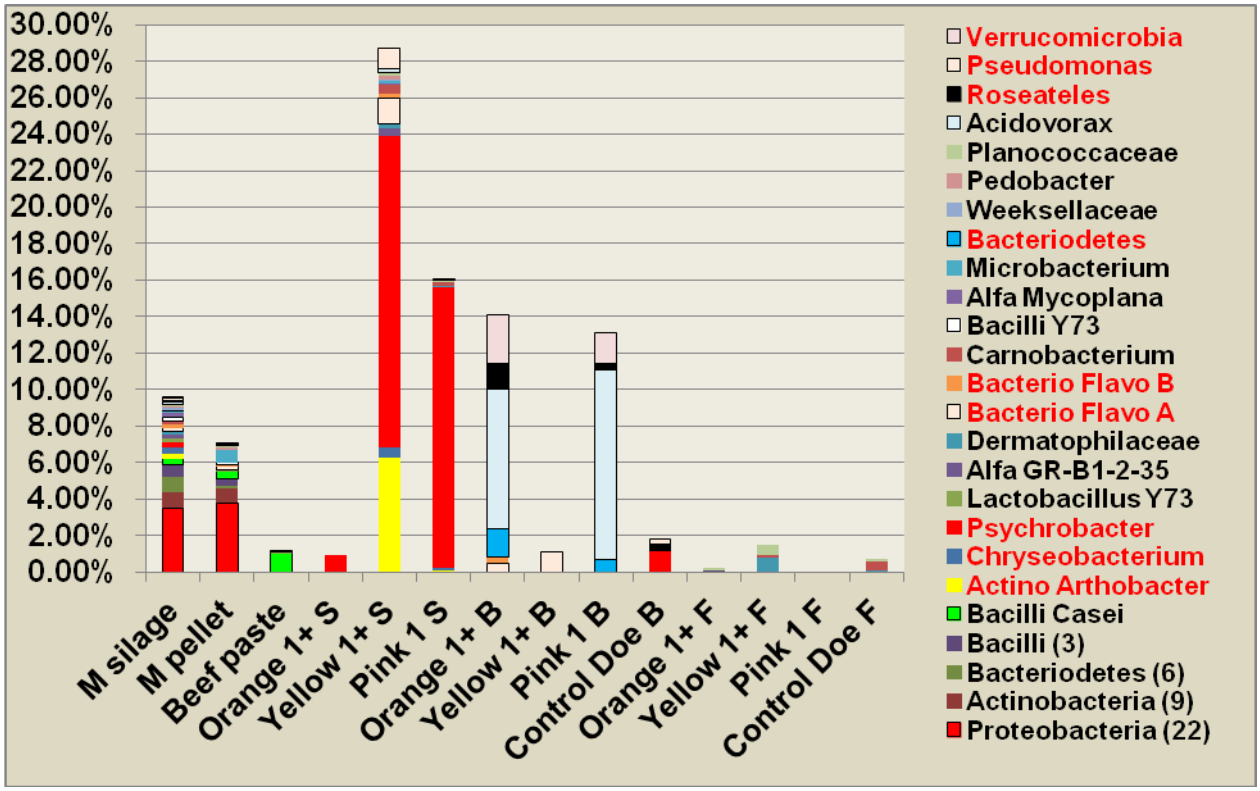


Table 5. Bacterial organisms (61) were identified (0.3% to 0.1%†) in the silage or silage pellets. There were 31 (red) organisms in silage products that showed expression in the saliva (S) of Orange 1+, Yellow 1+ and Pink 1 on the quarentined farm. Other negative bacterial organisms were found in the blood (B) of Orange 1+, Yellow 1+, Pink 1 and the control Doe's blood used in this study.

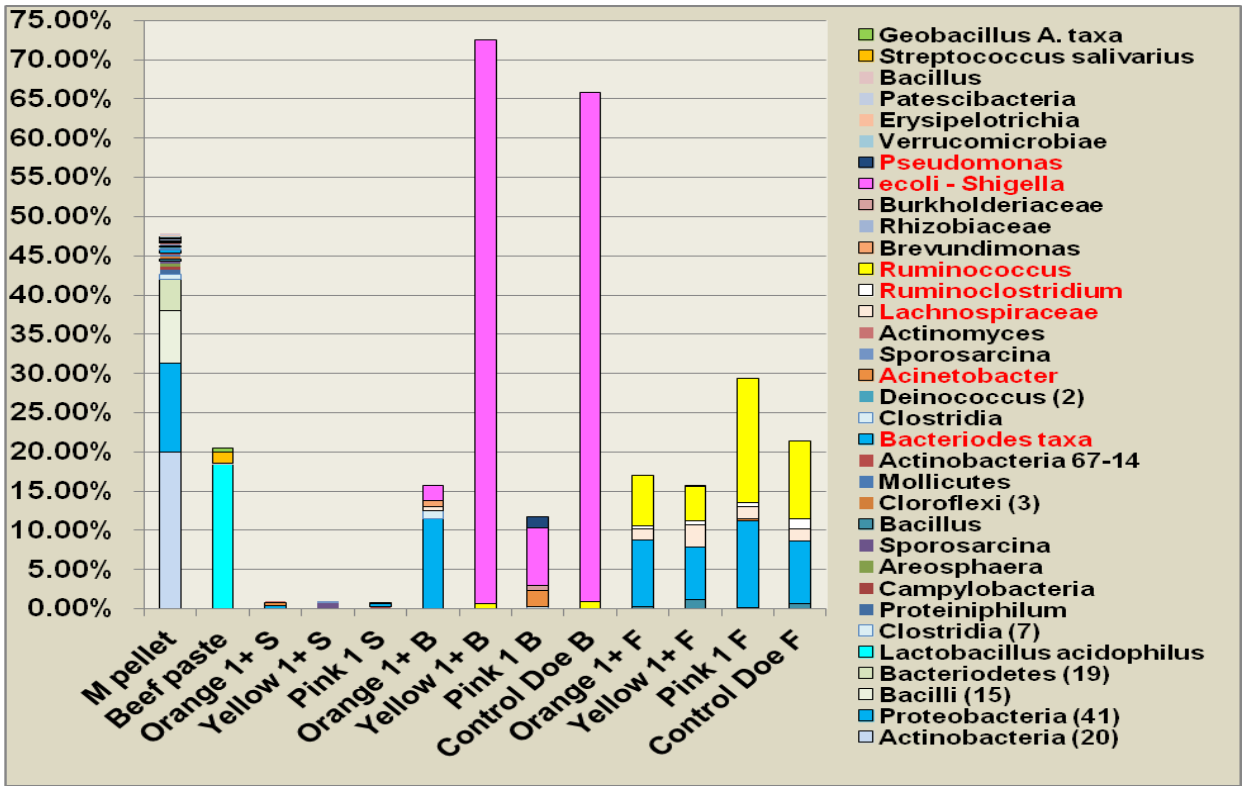
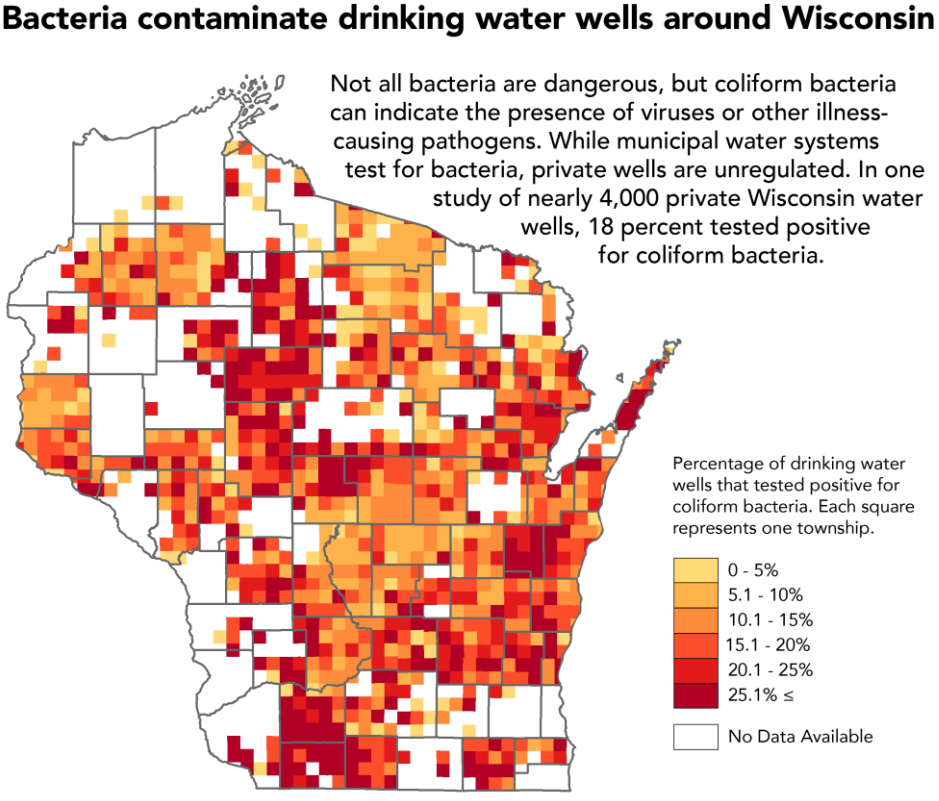


Table 6. The silage pellets also had 134 other bacterial organisms that resinated in the deer. There were 7 organisms (red) present in the silage pellet showing a presence in the blood (B) of Orange 1+, Yellow 1+, Pink 1 and the Control Doe. Multiple Proteobacteria (41) resinate from the silage pellet to organisms found in 4 of the deers fecal (F) content (blue) and in the blood of orange1 +.



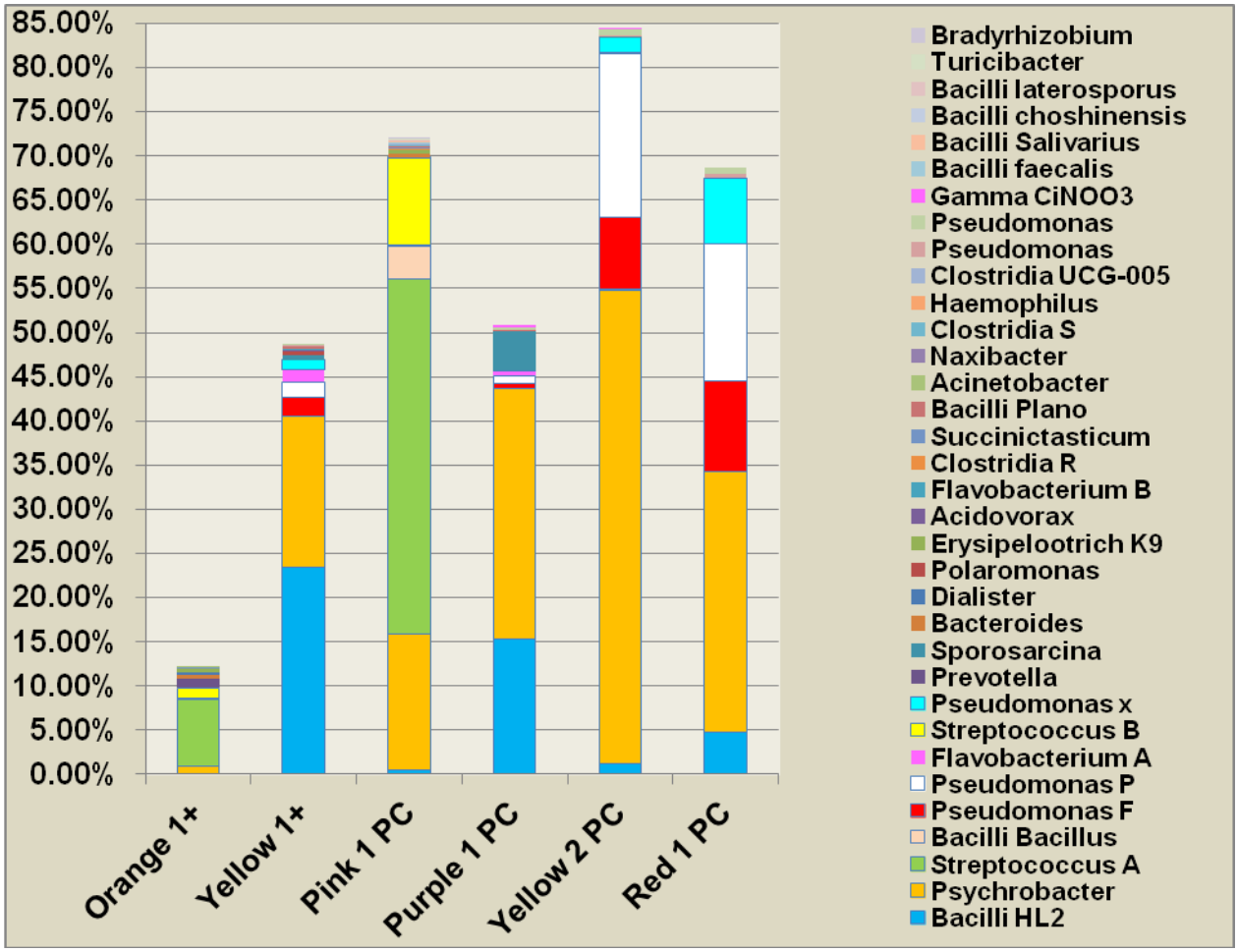


Table 7. Comparison of deer's saliva in the same pen consuming the same feed and water.

Though there are some conserved organism similarities amongst these deer (Table 7) there are differences noted in loss or change of saliva organisms in Orange 1+ and Yellow 1+ as compared with other deer (Pre - Clinical, PC) in the same enclosure. These changes could provide an opportunity to understand bacterial organisms for overall deer health or decline of respective saliva bacteria from certain dietary intake.

Silage, hay or other forage products produced for livestock feed under various conditions can harbor different levels of good and bad bacterial organisms. Feeding untested forages in an ongoing basis can, over time, lead to an increased negative health consequence for your deer.

Fecal – Parasites

These deer on the test farm were wormed in the summer of 2017 after a group fecal was collected and determined by fecal flotation to contain only stomach worms.
In a follow up fecal collection (fall 2017) the herd was parasite negative.
In the spring of 2018, separate fecal samples were collected from each deer for parasite presence. Results showed no parasites were present at this time of testing.

FECAL BACTERIA

Tested silage pellets, a different set of bacterial organisms were identified that resinated in the deer.

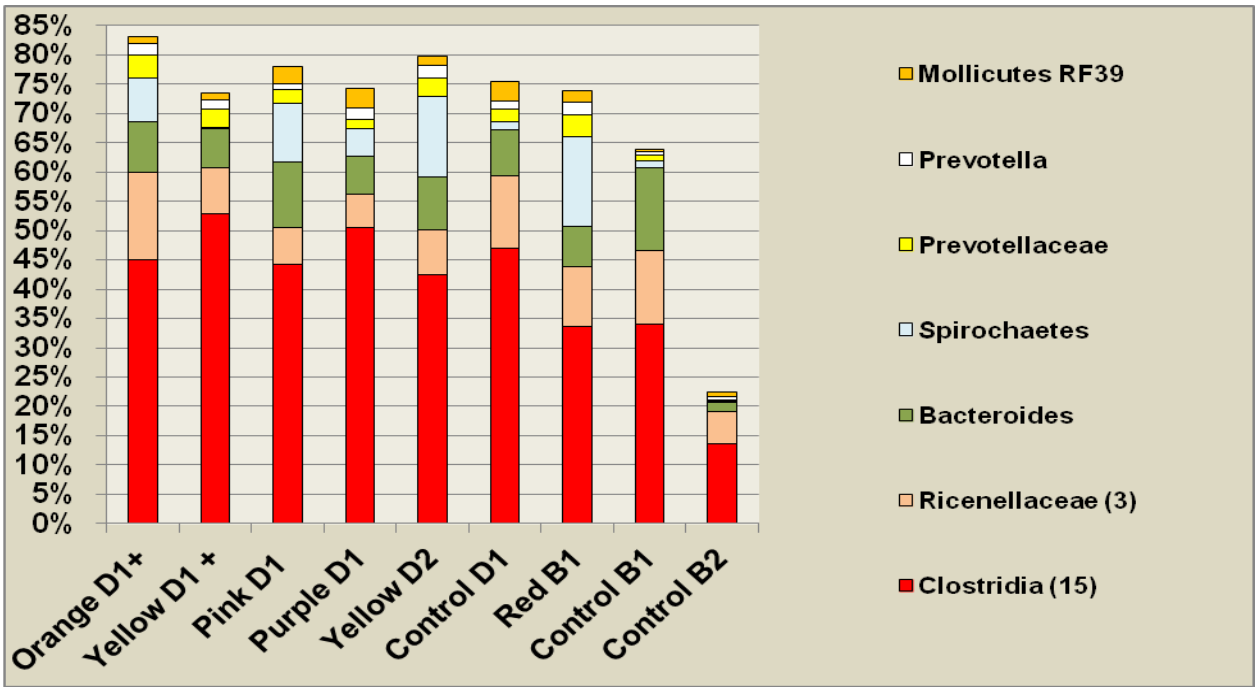


Table 8. Bacterial organisms (23) were identified (53% to 1%†) in the fecals of deer. Clostridia organisms (15) were the most abundant in all deer tested. Control buck 2 (B2) had reduced organisms as compared to other Buck or Doe fecals. Spirochaetes were absent in Yellow 1+ or reduced for control Doe 1(D1), control bucks (B1,B2) as compared to other deer.

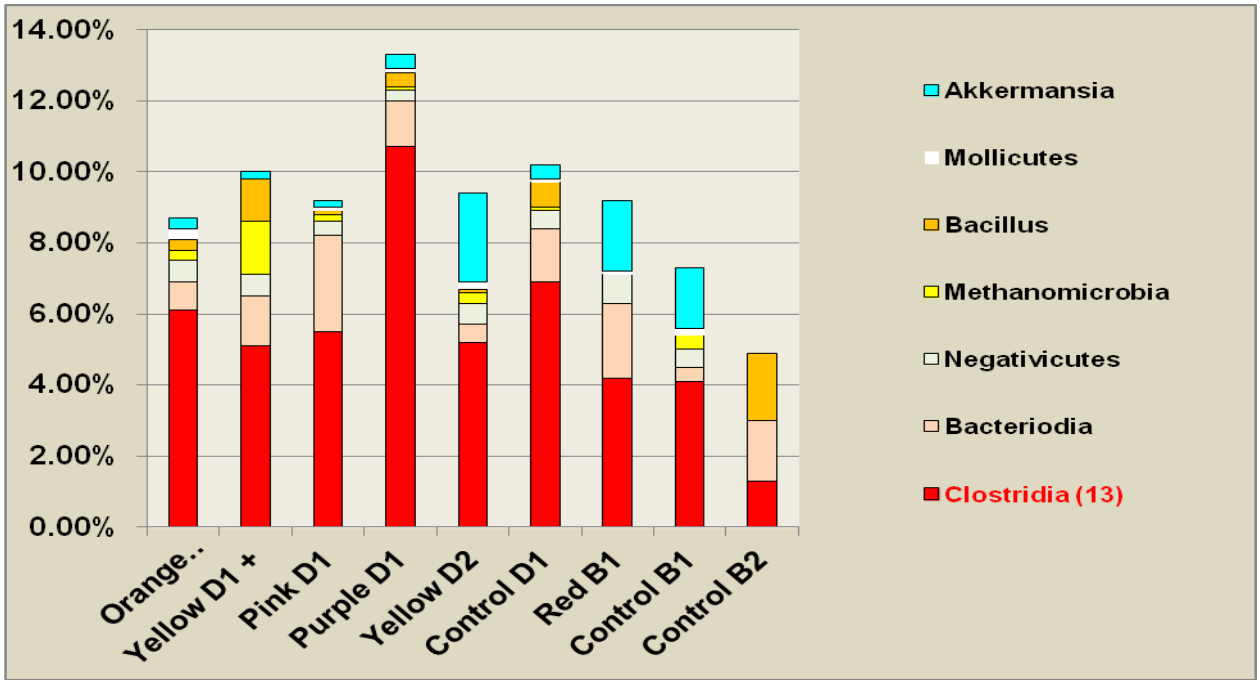


Table 9. Bacterial organisms (19) were identified (0.8 % to 0.3%†) in the fecals with 13 different Clostridia organisms being in the most abundance.

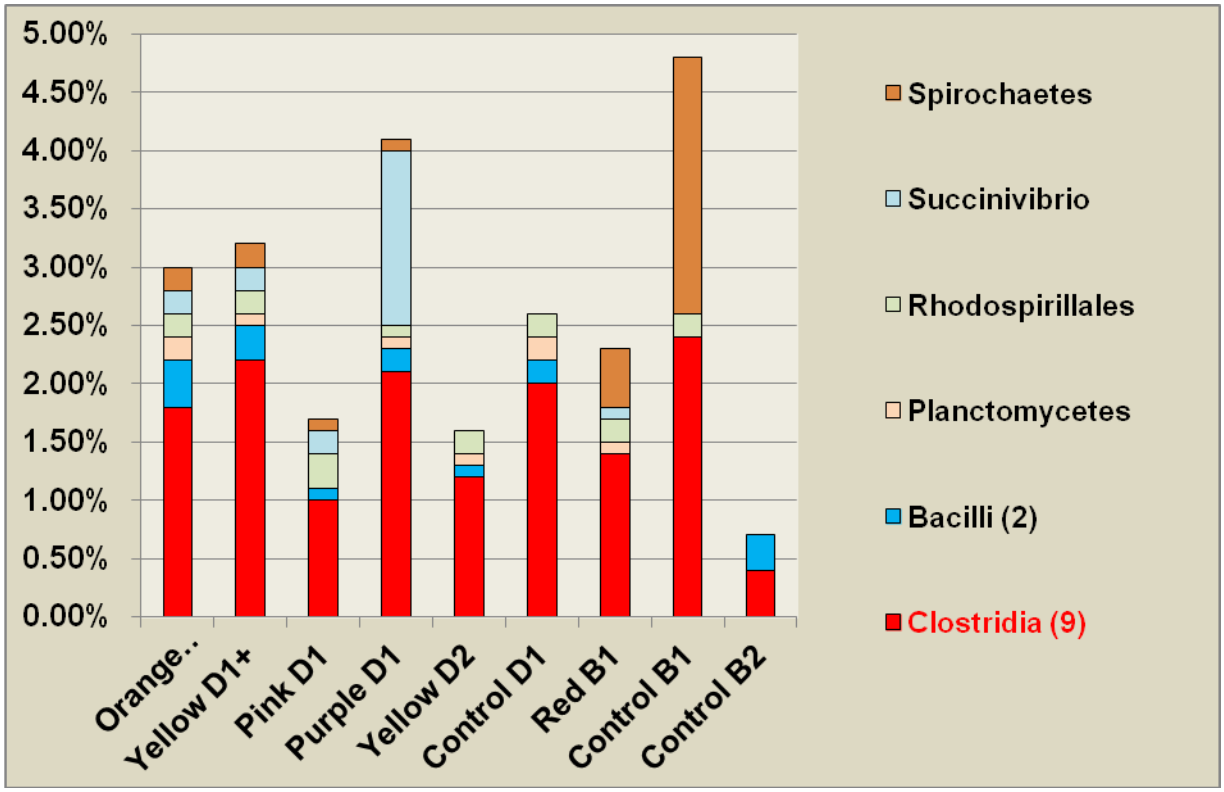


Table 10. Bacterial organism Clostridia (9) were most abundant identified (0. 2 %†) in fecals.

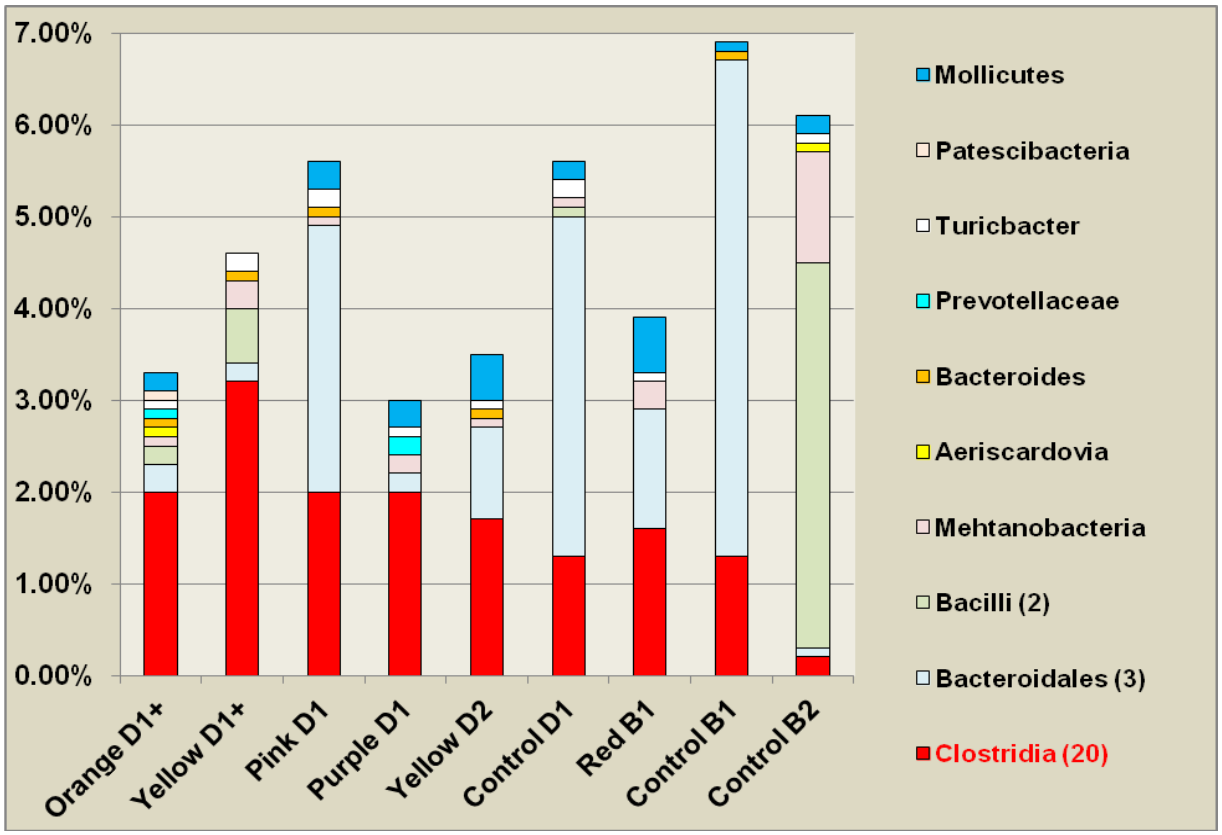


Table 11. Bacterial organism Clostridia (20) were most abundant identified (0. 1 %†) in fecals.

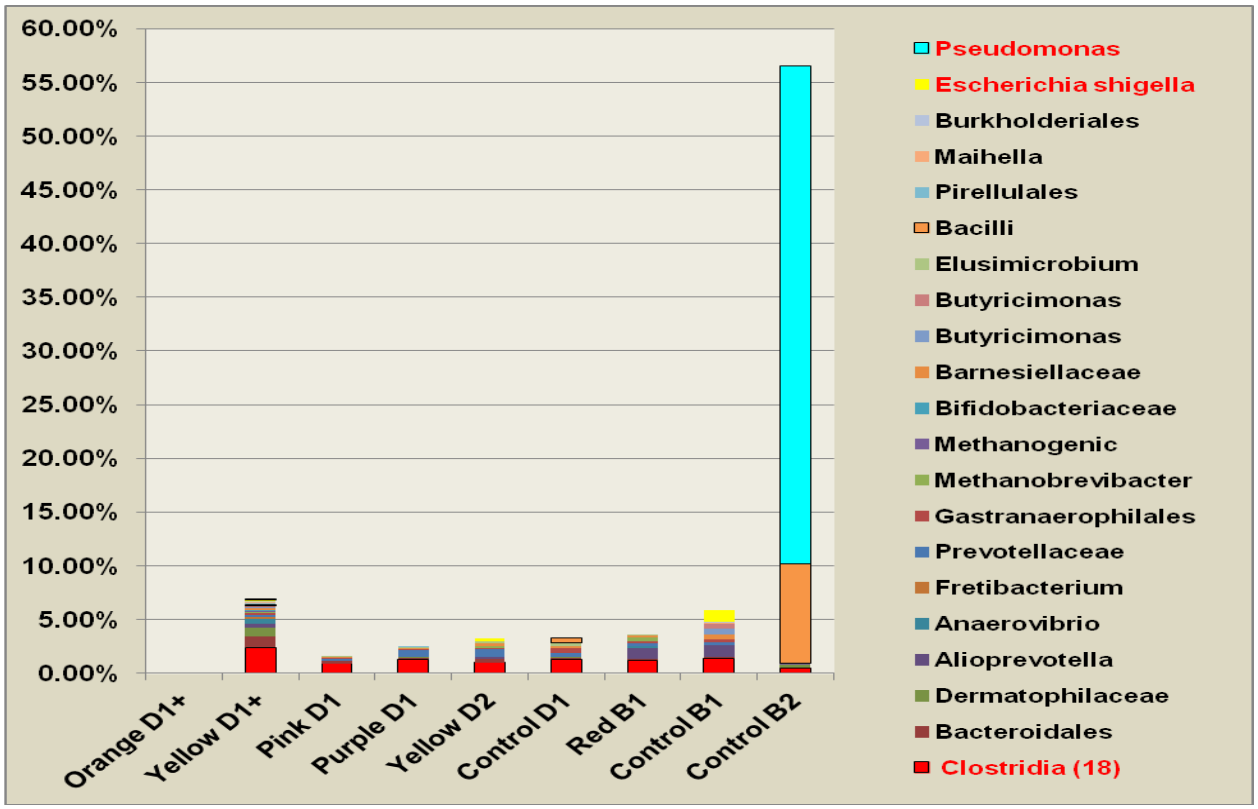


Table 12. Bacterial organisms (38) indentified in fecals of deer but not in deer orange 1+. Other fecal organisms in red or aqua are of concern for deer health.

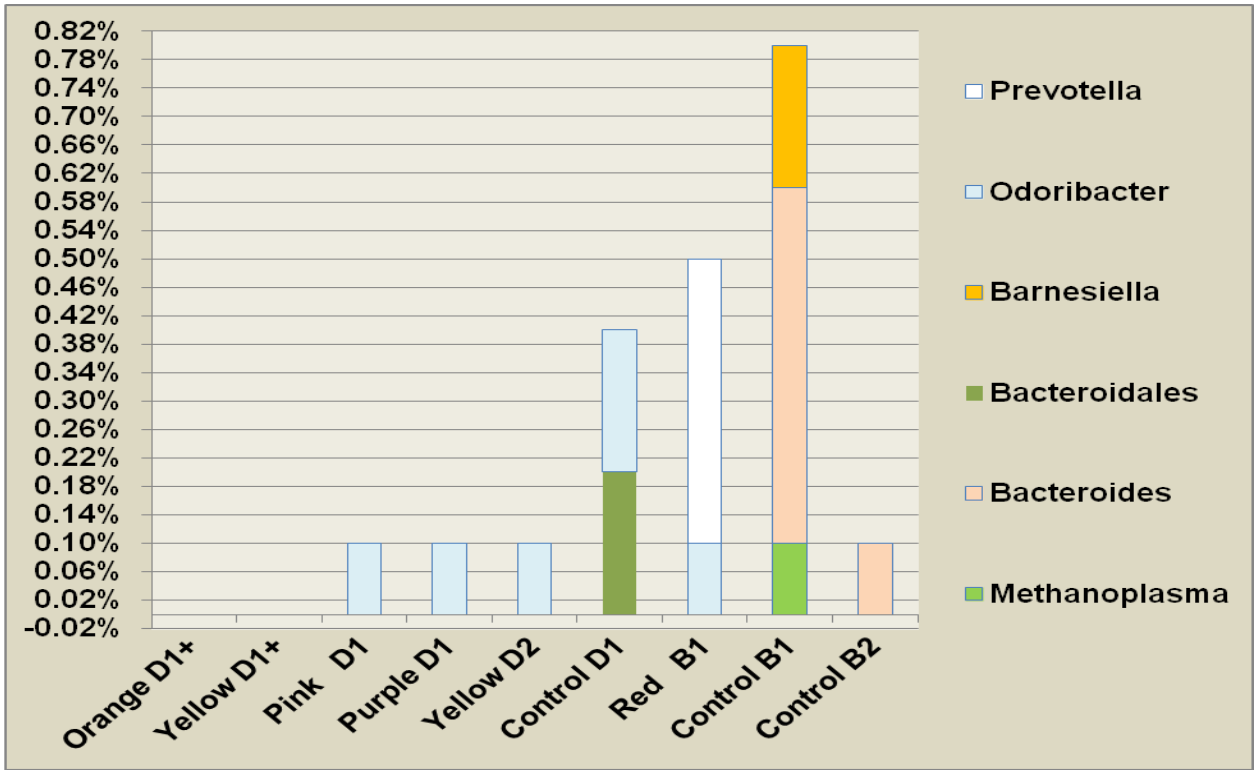


Table 13. Bacterial organisms (6) in other deer fecals not associated with orange+ or yellow+.

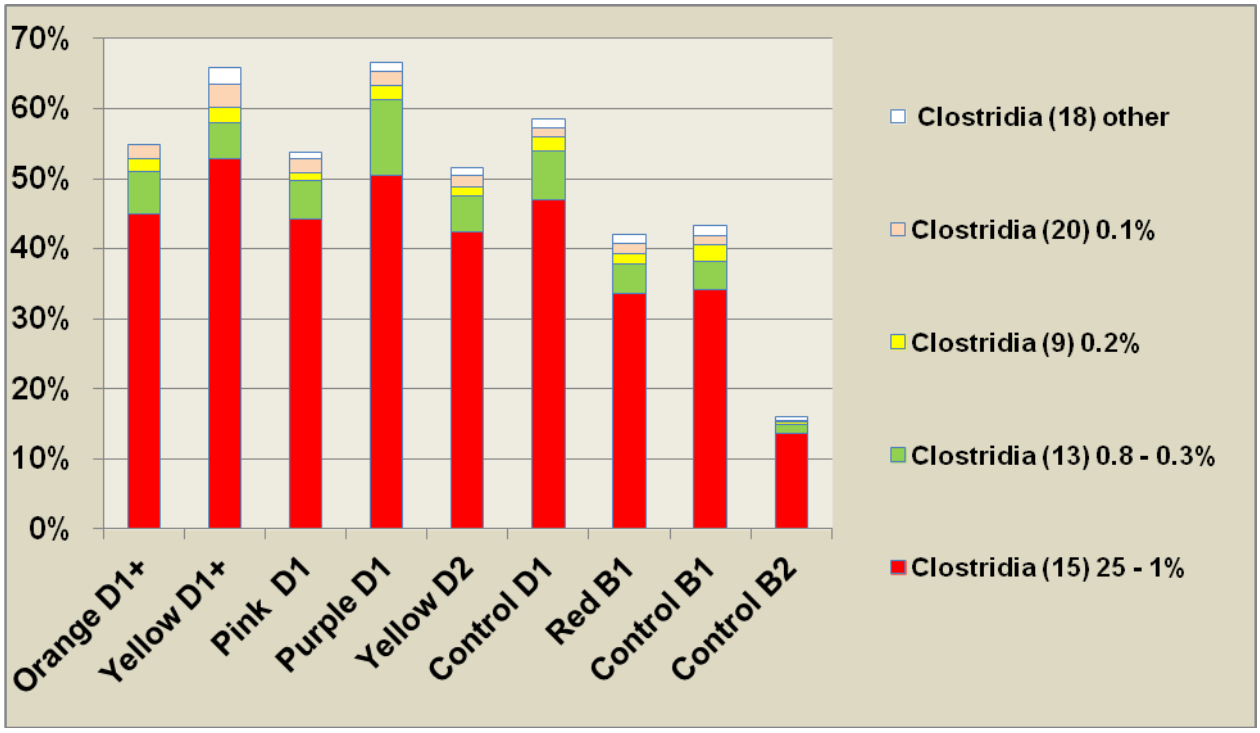


Table 14. Summary - Clostridia (75) were the most in abundance of all fecals of deer tested.

Blood – Pre Clinical

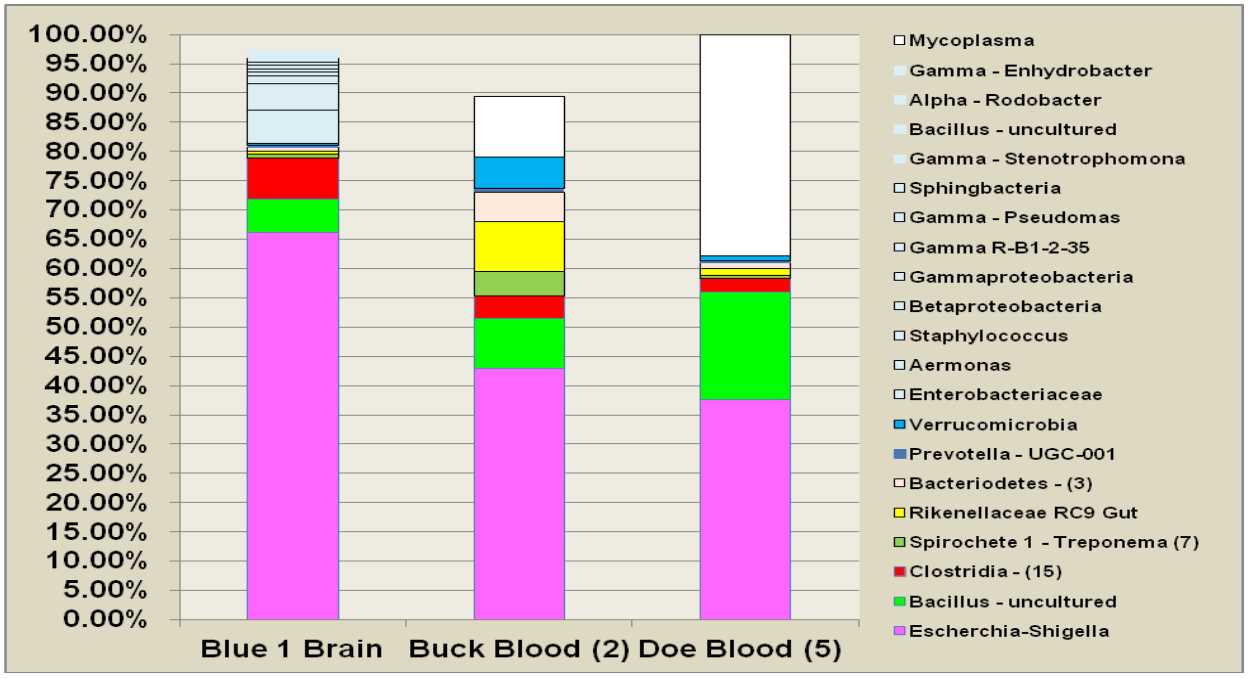


Table 15. Original pre - clinical blood and brain tissue provided by deer on the quarantined farm provides a basis for future comparisons to an onset of a disease process. Shigella was the most prevalent organism in both the blood and brain followed by uncultured bacillus and clostridia organisms. Mycoplasma was the only organism found in the blood (white) of both the 5 Does and 2

Bucks on the quarantined farm but not the brain of the deceased buck Blue 1. Other organisms (colored light blue) were unique to the diseased buck's brain that was later determined by IHC to be CWD negative by the USDA (2 x rectal, 2 lymph nodes and obex) testing protocol.

Doe Blood – Follow up to Preclinical Sampling

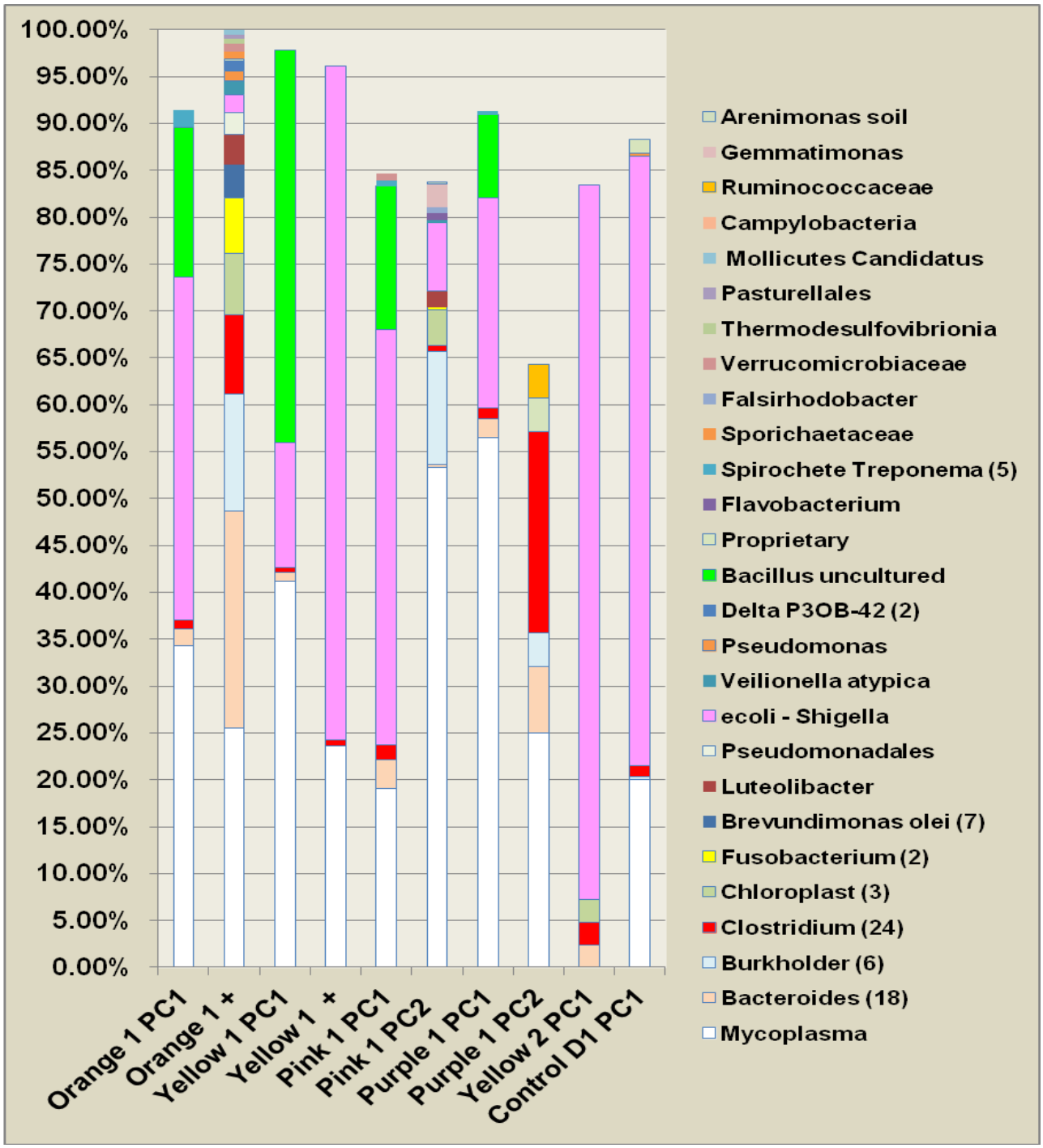


Table 16. Doe blood was collected for post clinical bacteria content and compared with pre – clinical bloods for underlying health status changes. (PC1 = pre-clinical blood 2016, PC2 = follow -up blood 2018, Yellow 2 and Control D1 = PC1 / preclinical blood 2018). Orange 1+ and Yellow 1+ = blood sampling from positive rectal biopsied deer (3rd biopsy).

Buck Blood – Follow up to Preclinical Sampling

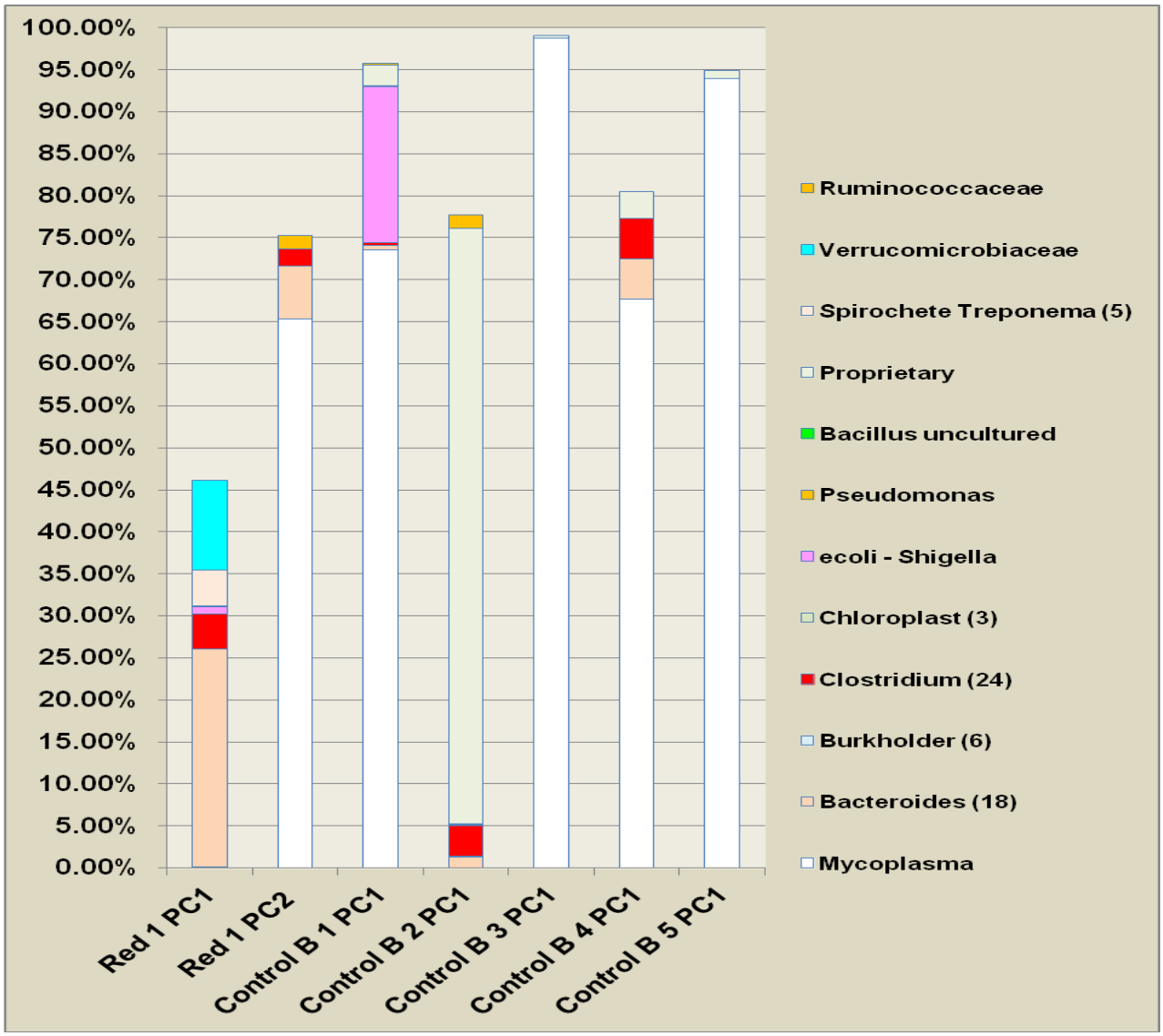


Table 17. The buck's blood (Red 1) was collected for post clinical bacteria content and compared for underlying health status changes along with other control bucks. (PC1 = preclinical blood 2016, PC2 = follow - up blood 2018, Control bucks used (Control B1 – B5 = PC1 / preclinical blood 2018).

The PC2 blood for Red1 shows a change in status in the updated blood sample results to include mycoplasma bacteria. The percentage of contamination is close to same as found in other control bucks used in this study as well as bucks from other farmed deer from out of state. The only exception was from control buck 2 (PC1) a 3 year old deer. Could this status change be worth following as when and how a deer acquires blood mycoplasma? Yellow 2 in the Doe group (Table 16) does not show this contamination after her second year in the herd as a 2 year old herd deer. Other herd Does show a positive sampling. Could this be an age related acquisition of mycoplasma since Red 1 buck also as a yearling herd deer did not show this organism in his blood? If this is found to be an acquired bacterial organism then one must review where and how this happens and if it is causal for other potential negative impacts to deer.

Discussion

The Phase 2 research was conducted to continue expansion of the initial findings of the Phase 1 study. This supporting information to the deer farming community is an effort to understand all health aspects of raising your cervids.

Our primary objective was to determine the CWD status of our quarantined deer by the use of rectal biopsy. This would be the 3rd time for the deer to be tested rectally for their CWD status. The deer sampled were noted (Table 1) that we now have 2 rectal positive detects. With 2 rectal positive deer (Orange 1+ and Yellow 1+) now on board, we can continue to monitor this disease progression. Taking pre-clinical samples before the onset of any disease process will provide a more accurate assessment of physiologic changes that are noted in disease progression. Using our pre-clinical data, as a bases for time duration to the onset of a positive rectal sampling, shows we are 980 days since the last positive detection in this small herd. This is a longer timeframe since Orange 1 was a GG genotype where as Yellow 1 is a GS genotype. The only difference is that orange 1 is 2 years older than yellow 1 (8 vs.6 years).

Hair was collected to test for new genetic markers for the deer on our quarantined farm as a review of genetic markers for new resistant genes towards the early onset of a prion disease known as CWD. The results showed that we do not have any special genetic resistance in the 6 deer on the quarantined farm.

Fecal assessments were also performed showing a continued negative test results for parasitic infection from the previous fall. One round of worming was done through the feed the prior summer for stomach worms.

Phase 1 results covered pre-clinical sample collections and analysis to include water, feed, fecal, parisitology of the deer on 3 farms. Subsequent pre-clinical research samplings provided by AOS were for forages, saliva, probiotics, blood, urine and tissue samples (lung, liver and brain) from both farmed deer and wild deer.

The pre-clinical and follow up samples were helpful in determination of future proposed research as to best materials for collection in a refinement for expediting areas of concern in early onset of disease detection, identification and or potential for intervention strategies.

Supplemental findings of the Phase 1 study demonstrated that there are certain gram negative bacteria that were detected in biological samples (blood, brain). Refinement of using blood sampling, noted a crossover of certain gram negative organisms residing in either the water, feed or other environmental exposures on all 3 farms as well as initial samples collected from a wild deer living in close proximity of the quarantined farm.

The Department of Natural Resources (DNR) has an ongoing research project for whitetail deer in the same area as our quarantined farm called the “Southwest Wisconsin CWD, Deer and Predator Study” - Investigating the relationships between deer, predators and disease. (SW – Study)

In a second study, research supported by the DNR in the same area of our quarantined farm was to study the potential prion transfer of disease (CWD) upon the landscape via wildlife and deer use of salt based mineral blocks in the environment. The study was called “Mineral licks as environmental reservoirs of chronic wasting disease prions” was noted in the beginning of this report.

In a 3rd study, referred to by the DNR as the “Sick Deer Response Plan” calls for citizens of the State to call in any sick deer they have come in contact with, as a surveillance tool for determining possible health issues with sick deer, or as a surveillance tool for monitoring the spread of CWD in the State as a whole.

In the SW - Study conducted by the DNR, the only disease concern for deer was for CWD identification (rectal biopsy) or the potential spread of CWD on the landscape when a deer died. Further post clinical information of this DNR study provides the opportunity to compare what is found in the wild deer in the endemic area as to what is found on the test farm, as it is located in the same high prevalence CWD area of the state.

In the necropsy reports of the SW - Study first year deer deaths, noted that “NO” deer died from CWD. Of note however were necropsy reports for 21 deer that were diagnosed with a variety of other disease type organisms as causal for diagnostic deaths of the deer besides predator death.

Upon further review of the DNR necropsy reports, it was noted that of the 21 reports received for dead deer in the first year only 6 died with detectible CWD. There were NO clinical cases where the pathologist noted the cause of death as a clinical case of CWD. The pathologist recorded causes of death from a variety of means from organisms responsible for diagnostic death (brain abscess meningitis, trueperella pyogenes (lung, brain), ecoli, clostridia, coccobacilli, pasteurella, multiocida, necrotizing broncho pneumonia, severe pleuro pneumonia, broncho pneumonia, severe pneumonia, moderate pulmonary atelectasis, enteritis or enterocolitis).

Other documented complications found during necropsies, which contributed to causes death were lung worm, nasal bots, and peritonitis, severe atrophy of fat, sepsis or poor body condition.
Only 3 deer were from noted predator cases, 4 deer of unknown causes, 1 from a car, 1 from a dog and 1 from a broken back. With these findings there was no pre-clinical assessment provided for any deer entered into the study when captured and radio-colored, other than rectal biopsies for CWD status before being released upon the landscape.

The second DNR study concerning the trace mineral blocks asserts that the deer population, concentrated by the use of trace mineral blocks, spreads CWD on the landscape and is with question. A multitude of wildlife and livestock visiting the mineral licks shows a wide variety of other animal species use the mineral licks for dietary supports in an ongoing basis. These results show that though other wildlife and livestock are not hindered by the onset of the TSE disease called CWD they could very well be consuming and or transmitting the prion upon the landscape as wildlife are not contained behind a fence as farm raised deer. The study also shows detected prions in fecal material of wild deer on the landscape. By only testing whitetail deer fecal material and not that of other wildlife species and livestock visiting these trace mineral licks provides results that only deer have detectable prions in their feces but only upon amplification. Since this is in an area of the State with the highest prevalence for CWD prions on the landscape (20 years) this finding of deer with prions amplified in their fecal material is not surprising.

The “Sick Deer Response Plan” data received to date only noted that of 127 deer were submitted in the first year of the study with 34 deer being detected with CWD when submitted for analysis. A request for the medical records of these “sick” deer shows that all deer were harvested and tested only for the presence of detectable CWD. There was no other pertinent health information generated for any “sick” deer submitted by the DNR as provided in the SW – Study deer necropsy reports. By only testing submitted deer in the “Sick Deer Response Plan” for CWD testing did not provide insight to other diagnostic health issues of why the deer were sick. This missed opportunity for the DNR to provide vital information as provided in the prior mentioned necropsy reports (SW – Study) would have allowed for comparable declining health information for our current study in identifying why 94 deer identified by citizens died without detectable CWD.

The information review of the 3 DNR studies is important information when complete and would be helpful to the Cervid industry and wildlife scientists alike in an effort to build scientific information in the interest of finding a resolution to any disease process.

Since our initial research started with the review of pre-clinical assessment of the quarantined deer and control deer our findings will provide for a more complete picture of any potential disease progression identified in deer. Conducting a study without pre-clinical assessment or including a control in the study would not be a prudent way of documenting a quantifiable research endpoint regarding the progression of a disease process in deer. This is noted in the necropsy reports as the wild deer having been diagnostically identified as dying from other disease organisms as found in deer rather than from CWD.

In the results, review of feed and water qualities offered to deer on the test farm noted that the quality of these feed stuffs do harbor negative organisms. These negative organisms do show that they transfer from feed to saliva of the deer. Once these organisms are on board with the deer they also show up in other areas of the deer’s digestive process (feed, blood, fecal). Review of the deer’s saliva is important to discern host organisms and/or changes to host organisms of the oral cavity. Cervid farmers have a vested interest in this area because they deal with various oral disease processes in raising deer costing them time, energy, medication and at times the unfortunate loss of deer.

There were other negative organisms that resonated more in the fecal materials of the deer on the quarantined test farm vs. the control farms. The deer on the test farm have never been vaccinated for clostridia species and but it is known that multiple clostridia organisms occupying the lower GI system could have a deleterious effect on the deer and their health status. Vaccinated control deer tested lower clostridia loads but it is unknown whether the vaccines are working accordingly in eradication of clostridia organisms. Continued monitoring of these and other bacterial organisms unique to Orange 1+ and Yellow 1+ considered positive detects vs. the other deer in this study certainly is warranted.

In review of the preclinical blood, results of the Does as compared to the current blood, shows the same basic organisms; mycoplasma and ecoli shigella as the leading organisms including a Doe used as a control for this study. Though individual farms on specific feeds, water and forages tend to be somewhat similar, there are differences between deer as individual animals. These differences do show a varying degree of organisms in their bloods but there seems to be a consistency towards negative organisms being present from younger to older deer.

Review of the pre-clinical blood results of the bucks, as compared to the current blood; show the same mycoplasma organism as in the Does but lacks the ecoli shigella organism in the bucks, with the exception of one control buck used in the study. It is interesting to note that, in their first blood panel as yearlings, Red1 buck and yellow 2 Doe on the quarantined farm did not have mycoplasma. Now Red 1, as a 3 year old, has mycoplasma expressing in his blood sample. Other bucks from multiple farms were also reviewed for various degrees of pre-clinical health assessments. Of the 5 bucks used for blood review 4 of the 5 showed the same mycoplasma in their pre-clinical blood review. Only one buck showed the ecoli shigella as seen in the deer of the quarantined farm. Another deer did not show any mycoplasma or ecoli shigella with a minimal of clostridia organisms in their blood samples as compared to other deer tested.

Conclusion

Since the deer were quarantined in early 2016 pre-clinical blood samples were retrieved and tested showing a certain level of gram negative bacteria in their initial assessment. In the sampling of the deer in the spring of 2018 (2 years) after being quarantined for CWD shows that these negative organisms continued to increase to a point where there was a negative health impact. Orange 1 and yellow 1 became rectally positive (+) for CWD after all deer having 3 rounds of rectal testing.

By generating new information as compared to past and ongoing developing research by AOS, organism's expression of various physiological systems of the whitetail deer (saliva, rumen, lung, liver, urine, fecal, blood and brain) can now be reviewed for comparative animal health status positive and negative.

Progress made in the Phase 2 study design for collecting samples for testing continues to show a direct correlation and understanding of where negative bacteria reside in the deer with potential to create negative health consequences.

Hay or other forage products produced from fields that use livestock manure or human wastes for landspreading nutrients will accumulate these unwanted negative organisms into the forages that are fed to deer unknowingly and can cause health issues. Knowing where forages are coming from will help in the long run by reducing the sick animal syndrome that many farmers deal with today. The same applies to the water source for the deer. Testing and keeping your water source clean and uncontaminated will go along way in reducing the negative bacterial load in your deer.

Recommendations from the results of this study so far would be for any forage, feed or water source be tested before providing your deer an unknown potential to to hinder their daily health status. Developing a supplier relationship and developing a healthier Bio-Security Plan will ensure not running short on quality feed supplies.

This new information can help the cervid farmer determine vaccine status, parasitic status and provide an overall health profile to support the deer overall immune status and disease suppression. Some deer that were vaccinated for these organisms still tested positive for bacteria loads such as fusobacterium, clostridia and other negative bacteria.

A Phase 3 proposal for continuing follow up of the deer on the quarantined and control farms will be generated to ascertain continuing health assessments of the deer during the current disease processes of Orange 1+ and Yellow 1+ or any other deer that might convert to a rectal positive CWD status in this past year of review.

Submitted by: Jerome Donohoe

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