Phase 3.5 Report Yellow 2 Report

Phase 3.5 will cover the necropsy and associated test findings for deer Yellow 2 which passed on September 14, 2019. Other recent timeline preceding Yellow 2's death along with relevant information can be found by reviewing the Peck Farm Research Update Fall 2019 or as listed in other research reports starting with Phase 1.

This report will build on findings of the end point health status of 2 other deer (Orange 1 / Yellow 1) that died last year 2018. This additional information will build on our base knowledge of deer health while under quarantine in determining a root cause process of a deer developing a terminal illness called Chronic Wasting Disease. Currently, this illness, in the farmed deer industry, has been largely unabated by best management practices, diagnostic surveillance, and depopulation efforts of farmed or wild positive herds (1).

Yellow 2 was rectally tested for the 3rd time in the spring of 2019 and determined to be rectal positive by the USDA NVSL facility using IHC testing methods. In addition to rectal biopsy, ultrasound imaging was used to assess the possibility of pregnancy. Yellow 2 was confirmed to be pregnant, the first time, since her birth in May 2016. Proactive planning in the event of her death, considering positive results from rectal biopsy and confirmed pregnancy, a strategy was determined that included gross necropsy in an effort to provide important information in this case. Certain biological samples were planned for collection in diagnostic testing that will provide an important ongoing review of her health status up to and upon her death.

To provide a more in-depth comprehensive report from CWD positive samples from deer, a USDA Federal Permit is required for the possession, transport and testing of positive materials. AOS has secured these permits as well as federally approved facilities for this effort to continue in-depth analysis of CWD positive materials.
By utilizing our base pre-clinical findings and past data acquired for Yellow 2 the proposed testing requests for funding will provide a continuance and comparison of data along with other deer in this study both past and present from control farms enrolled in this study.

Left to right: Brad waits for the deer to get into position before darting sedation for collecting samples. Ray describes the procedures to take place with Representative Mursau while the herd veterinarian Dr. Amy Robinson prepares a Doe to see if she is pregnant via ultrasound technique.

We will be collecting multiple samples from deer in the spring, and importantly, assess their health and body conditions in relation to winter survivability. Spring is generally a time where deer farmers are looking to build their herds through proper nutrition and expectant fawning season. This spring, the blood values collected for the deer on this farm were of a healthier condition (Table 1) than perceived from past samples. This healthier profile is due to the change in providing the deer a new ration designed for improvement towards reproduction status in ruminants. In particular, the new ration was incorporated to reverse the lack of reproduction in these deer from past feed rations supplied by the farmer or commercially available feed. The new ration was started on this quarantined farm in the fall of 2018 before the breeding season. The same feed was started at the same time on the other 2 control farm deer that are enrolled in this continuing study.

As the season progresses from spring, summer and fall, we will look at chronological events of the seasons and environmental effects that could directly impact the health of Yellow 2. We will also compare the overall health status of the other deer on this farm compared to deer on the 2 control farms with respect to their body conditions and health status. This comparative health information will help our industry farmers determine the needs for their own deer herds, and could provide important information enabling them to read signs of ill health, resulting in earlier actions to support farm production goals. Current research has demonstrated that both differential susceptibility to CWD, and natural variation in disease progression, are moderately to highly heritable among farmed U.S. white-tailed deer (1). To that end, broader health monitoring should include animals which have had artificial insemination (AI) procedures designed to increase sales and have resulted in a broad mix of genetic variation.

Blood Values	Unit	Pink 1+	Yellow 2+ PG	Purple 1 PG	Red 1	Average
Glucose	mg/dL	307	206	256	229	247
AST(GOT)	U/L	107	64	85	87	86
SDH	U/L	14.8	11.6	16.9	10.8	12.4
Total Bilirubin	mg/dL	1.4	2.1	0.9	0.5	1.3
Cholesterol	mg/dL	47	31	45	36	38
Total Protein	g/dL	6.1	6.1	5.8	6.4	6.2
Albumin	g/dL	3.4	3.5	3.2	3.3	3.4
Urea N	mg/dL	31	33	28	31	31.6
Creatinine	mg/dL	1.7	1.6	1.5	1.2	1.5
Phosphorous	mg/dL	1.6	7.5	4.2	5.5	4.8
Calcium	mg/dL	8.4	7.9	7.7	8.3	8.2
Sodium	mmol/L	140	141	139	134	138
Potassium	mmol/L	4.1	4.7	4.4	4.5	4.4
Chloride	mmol/L	105	103	104	99	102.3
Bicarbonate	mmol/L	24	23	22	25	24
CK	U/L	274	63	146	95	144
Gamma-GT	U/L	42	20	44	43	35
Anion Gap mmol/L	mmol/L	15	20	17	15	16.6
Globulin g/dL	g/dL	2.7	2.6	2.6	3.1	2.8
A/G	Ratio	1.3	1.3	1.2	1.1	1.2
Urea/Creat	Ratio	18	21	19	26	21.6
Sodium / Potassium	Ratio 32	34	30	32	30	31.3
Nonesterified Fatty Acids	mEq/L	0.592	0.62	0.571	0.499	0.57

(Table1.) Metabolic Blood Panel of the 4 remaing deer on quarantined farm. Elevated NEFA above 0.8 mEq/L in plasma may be antagonistic to the immune system.



Left to Right: Yellow 2 - born on 5-20-16 (genotype 96/GG) was pregnant for the first time in spring of 2019. She maintained good body condition into late summer prior to birth of her first fawn (buck) Yellow 3 on 7-24-2019.


Left to Right: Fawn Yellow 3 lounges with Red 1 (sire) on a warm summer afternoon while Purple 1 cleans her first fawn Purple 2 born on 8-15-2019. This was Purple 1's 1rst fawn in 4 years time after being on the new feed from the prior fall.

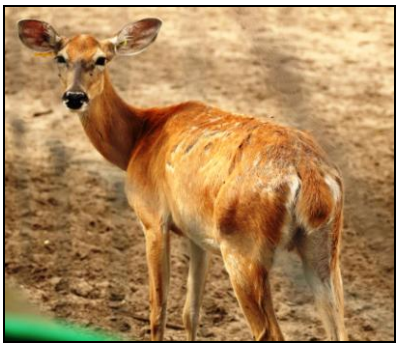
As we progressed into the summer months we were continually reminded of the many environmental conditions with seasonal changes and increasing risks for exposures to weather conditions, flies and bacterial organisms found around the farm which could impact your deer. The summer brought many thunderstorms and copious amounts of water making it difficult at times to maintain dry pen conditions.

As summer progresses to fall so do bacterial counts in the environment. The deer continue developing their velvet antlers ready to cast and Does begin to dry up on milk for the fawns. This is when stress usually starts to show up in deer. This stress could be compounded by many other conditions including pen densities, planned weaning separations or other deer and human movements on the farm in what would be considered normal farm activity.

Fawn Yellow 3 was un-expectedly found dead the morning of September 4rth. This fawn had a very vibrant disposition showing little course of slowing down. It was later found the fawn had an open sore on a back leg hock area that had become infected and covered with flies. A necropsy was not performed due to the state of decomposition at the time the death was discovered. From past observation, the next important time point for monitoring this disease process would be in the months

of August through December. These months signal the shortening days of fall that require deer to put on more body fat along with a new winter hair coat which critical for survival.





Left to Right: Yellow 3 was found dead unexpectedly on 9-4-2019 of unknown causes. Yellow 2 health starts to decline at an increased rate as the fall season approaches.

Yellow 2 passing on September 14th, 2019, Necropsy Report

A gross necropsy of Yellow 2 was performed 9-14-19. The deer's body was in good postmortem condition (A.) outwardly. The animal carcass was very thin with some hair loss on the dorsum, possibly postmortem change. Internally the loss of mesentery body (A.1) fat is a hallmark of the disease process.

Samples were collected for the regulatory requirement for CWD testing (obex and RPLN). Additional samples were collected for further diagnostic review in an effort to understand deer disease process leading to what we call CWD.

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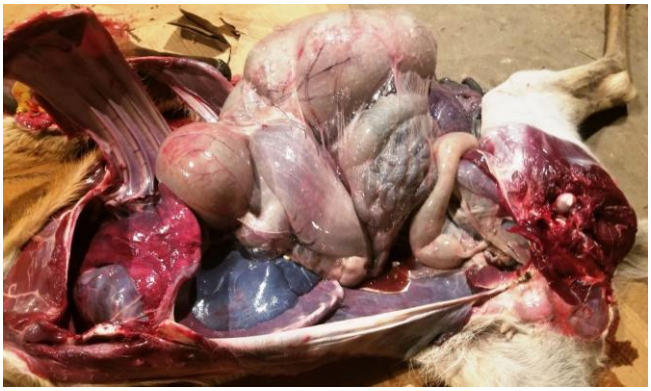
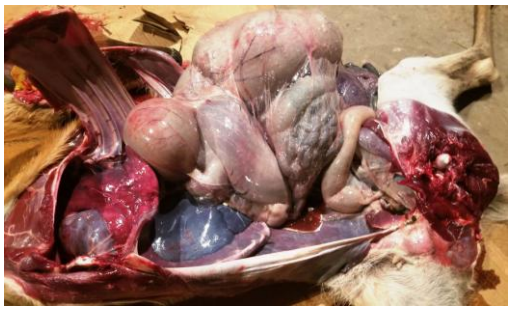
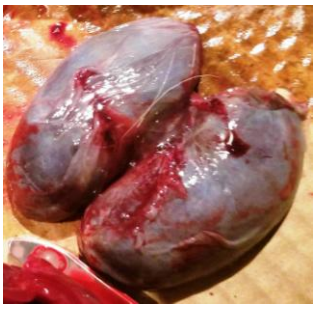
(A.1) 

Initial diagnostic results are reported as follows.

History - Farmed whitetail deer CWD+ by rectal biopsy. Tissues submitted for both diagnostic and research purposes. Obex and lymph nodes were submitted to NVSL for testing and confirmed positive in lymph node and obex.
Culture and histology of all submitted tissues requested, as well as fecal parasite evaluation and Mineral / toxicology panel. Sand impaction and torsion of cecum. Cachexia. No vaccinations or treatments administered.

Gross Findings - Received were numerous formalin-fixed and fresh tissues from a 3-year-old, female, whitetail deer (Yellow 2). The fresh lung specimen is diffusely dark red and wet. The mammary gland tissue is discolored green and appears autolyzed; it was tough with sectioning of tissue for submission for culture.
The rumen appears lesion free, and the abomasum is mildly reddened.
Formed fecal pellets are received. Additional fresh liver tissue is received bagged and specifically designated for element testing (liver submitted to MSU for referral testing). All fresh tissues were submitted for culture and feces were submitted for fecal analysis in house, as requested by submitter and client. The Wisconsin Diagnostic Laboratory was unable to complete a fecal flotation request due to CWD positive status of animal samples submitted. See full report attached.

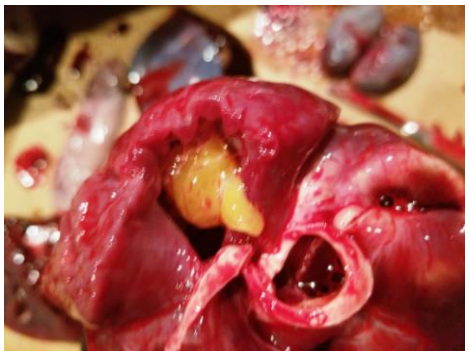
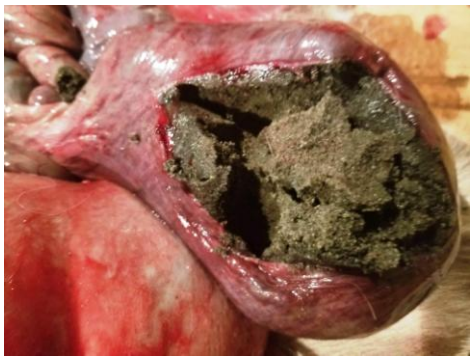
Upon opening the deer's body cavity for tissue collections it was noticed, as in past diseased deer, that little to no body fat (B.) was present internally. Specifically there was no observable fat in the omentum or surrounding the (C.) kidneys.

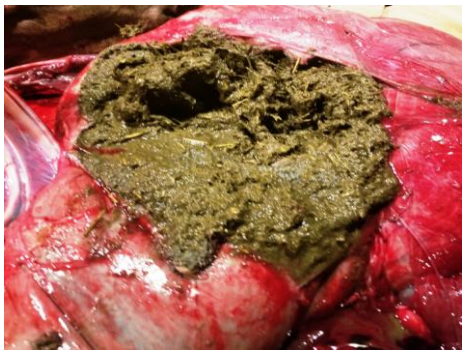
(B.)  (C.)  (C.) 

Upon review of the deer heart it was noticed a serous atrophy of fat around the heart with a prominent (D.) "chicken fat clot" in the right atrium. The cardiac wall was possibly thickened, but no measurements were taken.

There was a prominent sand impaction (E.) and torsion of the cecum. The rumen (F.) was full with normal digesta with the abomasal contents looked normal (G.) but a 3 cm impaction of feed material was occluding the pylorus.

There was a moderate amount of serous fluid (G.) present within the abdominal cavity with several large fecal balls covered with mucous were present in the colon. Blood samples were collected from large vessels opened during the necropsy along with from several body tissues for submission of fresh and fixed samples for testing review.

(D.)  (E.) 

(F.) 

(G.) 

There was no urine present in the bladder so samples were un-available for collection. The uterus was considered non gravid and was completely involuted from her previous pregnancy. There was milk still present within the mammary tissue though this deer seemed to have stopped lactating to her fawn in the prior days of her death. There was a mild inflammation in the udder tissue with a more severe autolysis present than in the rest of the body, possible signs of mastitis. All necropsy and tissue collections were performed by the herd veterinarian Dr. Amy Robinson DVM.

In table 2, there was 1 value (tan) that was considered above value ranges could be caused by either dehydration or fatty infiltration of the tissue. There were 7 values (green) that were considered in range that are considered typical for animals that are adequately nourished. There were only 4 values that were below the normalized range (pink) that are possibly due to possible over - hydration / electrolyte abnormalities.

	Results	Ref Range	
Dry Weight Fraction (Gravimentry)	0.342	(0.260-0.340)	
Heavy Metals	Results	Ref Range*	Units
Arsenic, Tissue (ICPMS):	<0.07	<1.5	ug/g dry
Lead, Tissue (ICPMS):	0.13	<1.5	ug/g dry
Mercury, Tissue (ICPMS):	<0.35	<6.0	ug/g dry
Thallium, Tissue (ICPMS):	<0.07	<7.5	ug/g dry
Trace Minerals			
Cadmium, Tissue (ICPMS):	<0.07	0.3 - 5	ug/g dry
Selenium, Tissue (ICPMS):	1.33	<6.0 - 13	ug/g dry
Iron, Tissue (ICPMS):	798.89	400 - 900	ug/g dry
Copper, Tissue (ICPMS):	16.02	180 - 500	ug/g dry
Zinc, Tissue (ICPMS):	145.86	75 - 210	ug/g dry
Molybdenum, Tissue (ICPMS):	1.16	0.9 - 1.8	ug/g dry
Manganese, Tissue (ICPMS):	14.72	4.5 - 10	ug/g dry
Cobalt, Tissue (ICPMS):	0.27	0.3 - 0.5	ug/g dry

Table 2: Liver sample submitted for Yellow 2 were compared to 297 Whitetail Deer Livers as noted in the data set provided by Michigan State University for heavy metals or macro/micro minerals.

The liver sample submitted for this deer shows various differences in the mineral composition as compared from 2 other deer liver results detected with CWD that had died on this farm in the past (Orange1 / Yellow 1). Deer going through the onset of a disease process seem to show independent differences in their mineral composition of the liver tissue.

The inconsistencies in liver minerals from deer to deer also show up in other deer tissue. In a past publication, the authors confirmed this by attempting to correlate liver mineral composition of lymph node and obex with CWD status: positive or negative (2). This group set out to established reference intervals (RIs) for these metals (minerals) and explored their ability to discriminate between CWD-positive and CWD-negative animals. Their results indicated that independent of CWD status, white-tailed deer accumulate higher concentrations of Fe, Mn and Mg in RLN than in obex. White-tailed deer infected with CWD accumulated significantly lower concentrations of Mn and Fe than CWD-negative deer. These patterns differed from other species infected with prion diseases. Overlapping values between CWD positive and negative groups indicate that evaluation of these metals in obex and RLN may not be appropriate as a diagnostic tool for CWD infection in white-tailed deer. Because the CWD-negative deer were included in constructing reference indexes (RIs), high specificities were expected and should be interpreted with caution. Due to the low sensitivity derived from the RIs, the group does not recommend using metal concentrations for disease discrimination.

Pathogenic organisms are not normally found in normal healthy brain tissue. The deer Blue 1 (10 year old) was found dead 282 days after the first index CWD+ case on the Peck Farm. Though this buck's brain material had a higher degree of shigella e-coli presence (Table 3), there was the underpinning of the areomonas pathogenic organism present. In Yellow 2's brain areomonas was greatly enhanced where as the shigella e-coli organism was completely missing. Other pathogenic organisms present in the CWD+ positive brain included Acinetobacter spp., Acinetobacter Iwoffii and B. myroides but not other organisms such as Bacillus, Enterobacteriaceae or Clostridia (13 types) as noted in the negative brain tissue.

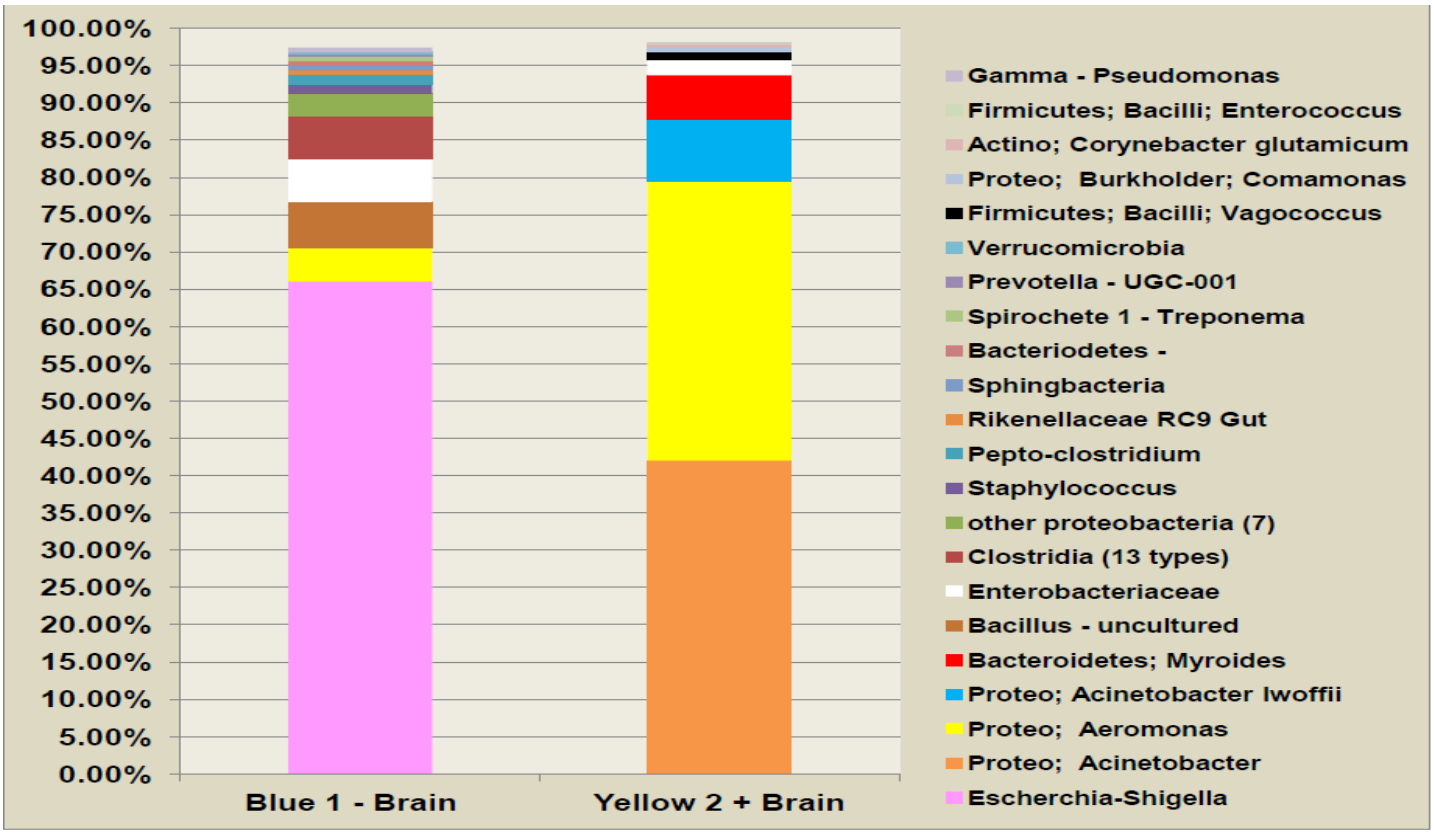


Table 3. Bacterial organisms of certified CWD negative brain tissue (BLUE 1) as compared to certified CWD+ brain tissue (Yellow 2) from the Peck Farm.

In table 4, these pathogens as well as others not listed are noted for their capabilities to disrupt the cells normal functional tasks of properly folding proteins. These various organisms through their pathogenic overgrowth increase in volume over time to take over the cells UPR mechanisms of normalized protein folding for their own survivability. This type of high jacking of the cells proper function by these pathogens results in increased cell stress both internally and externally by using various methods including toxin metabolites like excessive Lipopolysaccharide (LPS) production.

Bacterium	Virulence Factor	Cell Type	UPR-specific host response - Protein Assembly / Folding		
			IRE1	PERK	ATF6
Aeromonas hydrophila	Water born infection	All cell, HeLa	XBP1-s	n.d.	n.d.
Pseudomonas	Environmental	C. elegans (parasite)	XBP1-s	n.d.	n.d.
Shigella dysenteriae	Stx1	Monocytic THP-1	IRE1, xbp1-s	PERK-p, chop	ATF6 cleavage
Staphylococcus	Environmental	BMM, RAW264.7	xbp1-s	n.d.	n.d.
Streptococcus pneumoniae	H2O2	H441	decrease xbp1-s	PERK-p, atf4, eIF2α-p, atf3, chop	n.d.
Gram-negative bacteria	Cell toxin LPS	Monocytic THP-1	XBP1-s	PERK-p, eIF2a-p	ATF6 cleavage

Table 4. Bacterium found in the CWD+ brain tissue that are responsible for high jacking the UPR- specific host response mechanism for protein assembly and protein folding correctly.

In table 5, the pathogens identified show their mode of actions and locations where found at various points of entry on the deer's body, as well as the action they use to diminish the deer's immune system. When a deer acquires these pathogenic organisms and cannot clear them through normal immune system activation, it demonstrates the potential capability to vertically integrate these disease causing organisms to other deer. This was explained and was shown in past reports of this study that deer eating or drinking from the same food or water source / receptacle increase their potential of sharing of common bacterial organisms within a specific group of deer. This type of sharing negative organism's overtime will usually fester over a time period but will only typically be noticed in weaker deer during stressful periods of such as weaning, moving / mixing deer, switching feed sources, inclement or seasonal weather changes and shipping stress. On the farm or in the wild areas around the farm risk factors to be mindful of for shared pathogenic organisms would include using or sharing feed items or equipment having a direct or indirect crossover access with wildlife or other livestock species.(3,4,5,6). Sales or use of wildlife bait or feed practices as noted by the AFWA provides markets for surplus agricultural commodities considered unfit or unmarketable for human or livestock consumption should not be used due to known or unknown bacterial contamination .(8)

Bacteria	Action	Location
Acinetobactor	Immunity	Nasal, Oral, Blood
Proteo G Alcaligenes	Septicemia	blood, urine, Feces
Clostridia baratii	Cell Toxin	Blood, Urine,Feces
Mycoplasma	Immunity,Biofilm	Blood Bio-film
E. Shigella	Immunity,Biofilm	Blood / Tissue
V. Akkermansia	Mucosal degrading	Intestine

Table 5. Bacterial organisms and their mode of action / location creating a negative depressing effect on the deer's' immunity.

In table 6, only the proteo-bacterium Desulfobrio organism was found in the deer drinking water to any appreciable level (4%) but to a lesser extent in the deer fecal material. In contrast there were 16 organisms that averaged only 0.185% each in volume that resonated in all tissues samples tested from the deer. Usually it was the shigella – ecoli organism that predominated in the blood of the deer but now resonates in the oral cavities, lymph node and fecal areas. Staphylococcus sp. was now the predominate organism in the blood with other areas of occupation in the nasal or brain regions. The proteo-bacterium Comamonas and Pseudomonas sp. DQ-01 organisms were uniquely positioned in the oral and brain regions originating from the deer's drinking water source on the farm.

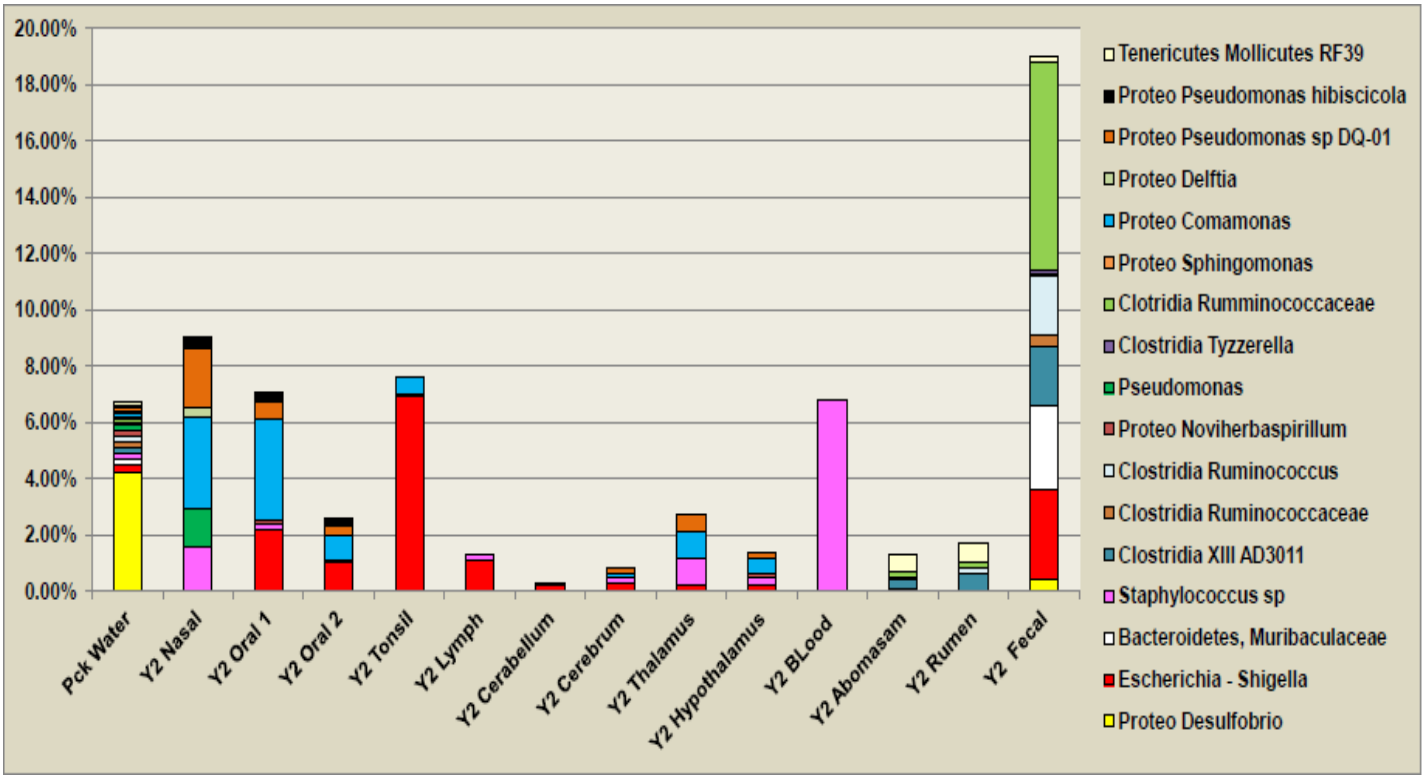


Table 6. Low amounts of bacterial organisms found in various locations of the deer that are associated with the deer's drinking water.

In table 9 shows bacterial organisms that are not associated with the deer's drinking water source on the farm. The leading 2 organisms Acintobacter spp. and Aeromonas along with others show the greatest presence in duel synergies located in the cerebellum, cerebrum, thalamus and hypothalamus.

The cerebellum and cerebrum portions of the brain were chosen and dissected for their specific location for testing is the same location where normal prion accumulation occurs. Pathogens associated with these 2 brain tissue centers will create an environment of the misfolded protein altering normal brain function over time. Areomonas was the predominate organism in these 2 structures. In the other 2 brain structures, the thalamus and hypothalamus are also a critical find with the presence of Acinetobactor spp. being the predominate organism. Acinetobactor appears to outline a pathway in starting with the nasal tissue, proceeding to the oral, and tonsil regions, then to the four critical areas of the brain, as well as detection in the fecal contents. The rumen only had a small amount of clostridia and mollicute organisms for which are considered normal organisms and connected to the water source.

What is compelling, however, is the lack of organisms typically found in the rumen (less than 4%). This demonstrates a loss of both diversity and volume of digestive organisms needed to digest the feed found in the full rumen upon death (Table 6, 9). This lack of rumen diversity and volume of normalized digestive organisms would support the comments in the necropsy report where changes in the rumen were indicative of some degree of acidosis and / or the presence of other mucosal irritating agents. This would be more supportive of mucosal irritating agents having access to the rumen that would have killed off the normal bacterial species typically present aiding in digestion. These irritating agents could be the same agents produced by the overload of gram negative bacteria over time producing the toxin LPS. This irritating agent pathway would be played out over time since acidosis in the rumen is typically caused by the inclusion of higher amounts of corn or other grains making up the primary content of the deer daily feed. The new ration used on this farm does not contain any corn products as other rations that are commonly used in farm feeds or large amounts of corn available to the wild deer population.

Acidosis in grain overload in ruminants is typically a short term condition that will kill off the rumen organism in a short period of time causal of ruminant death. In table 7 notes some of the metabolites produced in the rumen of a ration consisting of high grain like barley / corn in the ration. The leading metabolites methylamine (ammonia), ethanol and uracil increase dramatically with including high grain diets in the ration from 0% to over 30% inclusion rate (7).

Table 7.

Rumen Metabolic Pathways	
Increase	Decrease
Methylamine	4-Hydroxybutyrate
Ethanol	Isoleucine
Uracil	Lactate
3-Phenylpropionate	Aspartate
Hypoxanthine	Succinate
Ethanolamine	Phenylacetylglycine

Table 8.

Ration w/ Corn Product	Ration+ No Corn Product
Glutamic Acid	Glutamic Acid
Alanine - L	Alanine - L
Aspartic Acid	1. Lysine*
1. Lysine*	Glycine
Glycine	Aspartic Acid
2. Threonine*	2. Valine*
3. Leucine*	3. Leucine*
4. Isoleucine*	Proline
Proline	4. Threonine*
5. Phenylalanine*	5. Isoleucine*
Tyrosine	6. Phenylalanine*
6. Methionine*	Tyrosine
Histidine	7. Methionine*
Alanine - beta	Histidine
7. Tryptophan*	Alanine - beta
8. Valine*	8. Tryptophan
Arginine	Arginine
Serine	Serine
Cystine	Cystine

Dairy cows fed high-grain diets during early lactation have a high incidence of metabolic disorders. High-grain diets (>30%) result in increased rumen fluid concentrations of several toxic, inflammatory, and unnatural compounds including putrescine, methylamines, ethanolamine, and short-chain fatty acids. Hydrogen peroxide is also generated and known as an extremely toxic compound for a variety of eukaryotic cells and can cause oxidative stress, which may be associated with metabolic diseases. Uracil increases more than 100 fold with increases of grain in the ration and could limit the bio-availability of folate for normal cell function (9). If folate in a ration is limited, uracil incorporation increases and genomic instability develops. This condition develops as cells of the body attempt to repair themselves, breaking DNA molecules to remove uracil. When folate concentration is limited, the precursor pool of deoxynucleotide triphosphates is reduced, and misincorporation of uracil increases in “a catastrophic repair cycle” which may promote double-strand breaks, chromosomal damage, and abnormal cell growth. Other area of amino acid synthesis and availability is the reduction of valine (table 8). Valine is an important amino acid through dietary means for proper support of building muscle cell structure.

Using high grain like barley / corn in a deer diet could help explain some of the negative nutritional conditions that could lead to a declining health condition in the deer over time.

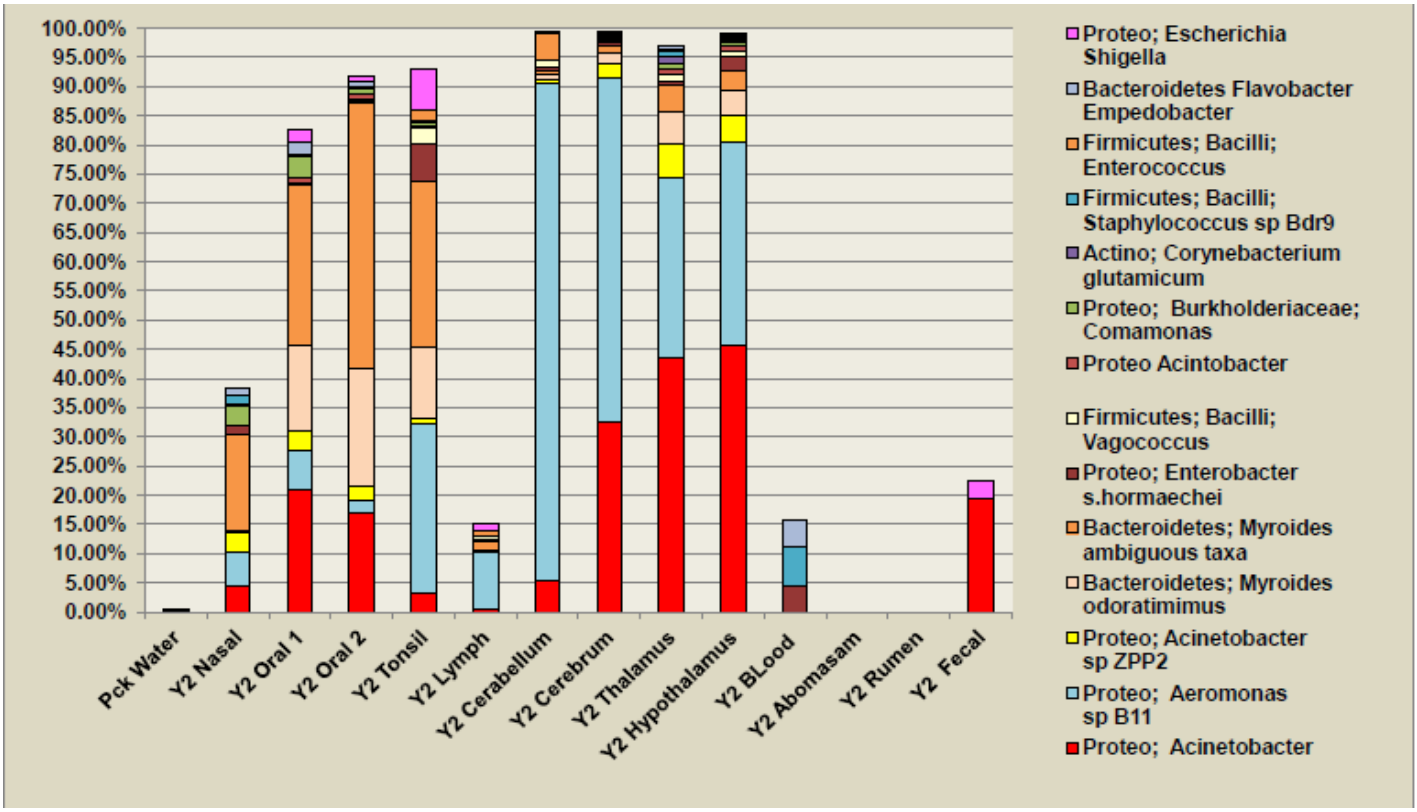


Table 9. Bacterial organisms found in various locations of the deer that are “NOT” associated with the deer’s drinking water.

Table 10 is a diagram of the hormone pathway starting with the hypothalamus that is located in the brain. This structure supports the mammalian body in normal biologic supply and control of hormone production and distribution throughout the body. The hypothalamus acts as the connector between the endocrine and nervous systems playing a part in many essential body functions. Through nutrition, these systems play a big role in maintaining body temperature, thirst, appetite / weight control, emotions, sleep cycles, hormones / sex drive, gestation / birth of offspring, blood pressure / heart rate, production of digestive juices, balancing liver mineral use and bodily fluids.

As the most important hormone control center, the hypothalamus controls the majority of the signaling to other regions of the brain and body for maintaining proper bodily functions. In Yellow 2 the hypothalamus was contaminated with multiple pathogenic organisms (Table 6/9) capable of causing the major disruption of normal hormone control elsewhere in the body. One area of concern found during the necropsy was the deterioration of the adrenal glands from both kidneys. This wasting away of the adrenal glands creates an imbalance and loss of hormone signaling control in the whole hormonal system of the body for proper growth. This also would explain the missing adipose fat from the mesentery area surrounding the intestinal tract. Though there are many different pathways to the adrenal glands from the hypothalamus the mesentery loss of adipose fat is through the hormone signaling through the liver function of chondrocytes. The liver is also responsible for production of growth factors supporting replacement of body muscle tissue, cartilage and other fat reserves.

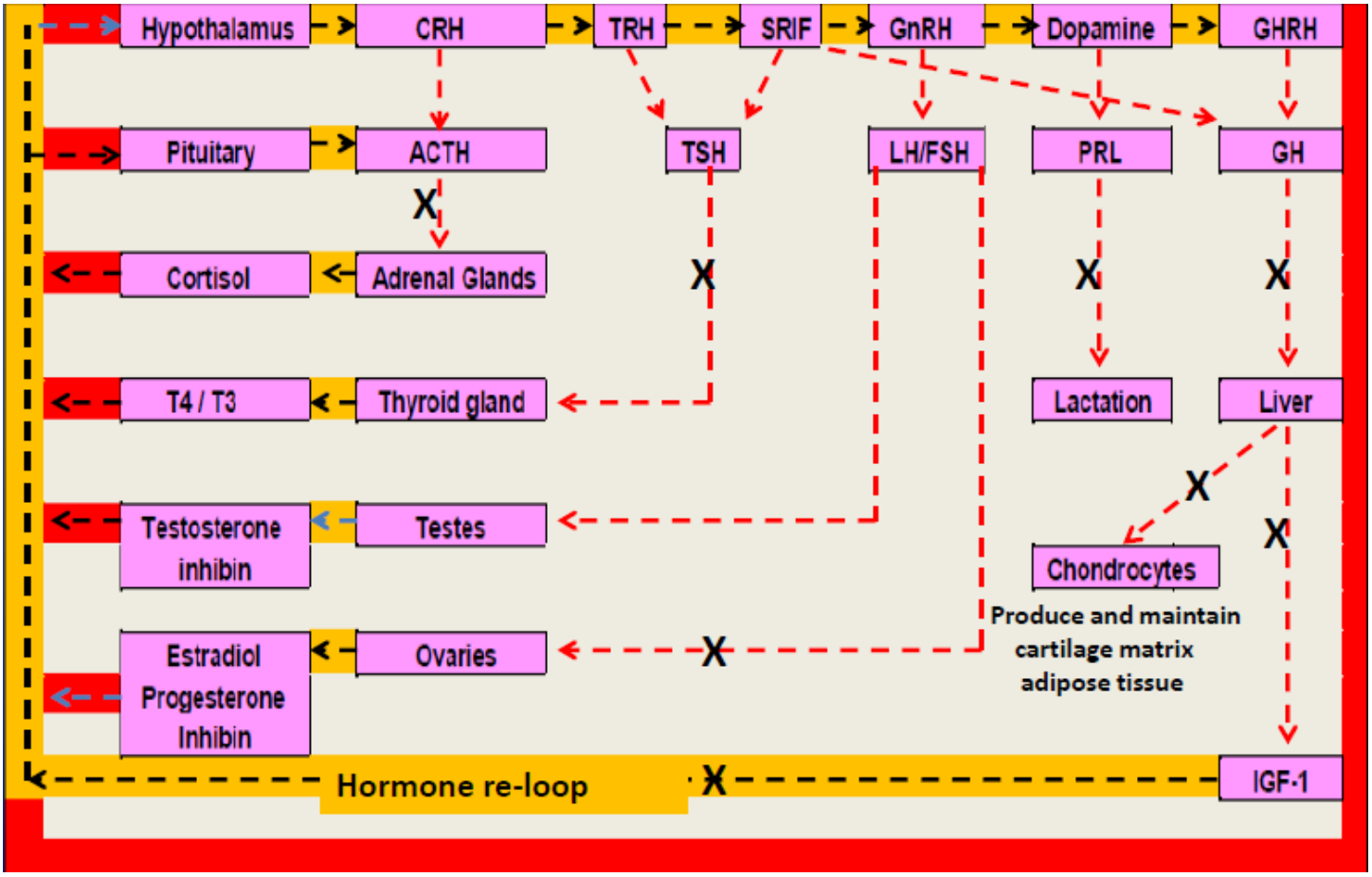


Table 10. Pathogens in brain control centers interrupt (X) key Hormone Pathways Leading to a "WASTING DISEASE"

Other areas of hormone function include providing for reproduction which is signaled through the testes and ovaries. One reason for switching from an industry supplied ration to the new ration without corn was to see if there was any interruption of any reproductive hormones in the deer enrolled in this study. When deer are under a nutritional or bacterial pathogen stress one of the first bodily function beyond maintenance that typically is spared would be reproduction.

The new ration for reproductive status was verified in the Control Farm part of the study by using 2 older adult Does not exposed to CWD where they had lost their reproductive status and not fawned for the 2 prior breeding seasons. Each of these Does on the new feed gave birth to twin fawns this past spring verifying that these older Does were nutritionally challenged lacking reproduction status on an industry ration that limited these two Does in their reproductive capacity.

Another component of reproduction portion of the new feed would also look to see if the buck was intact to be able to produce fawns with the Does. The buck produced 1 fawn with Yellow1 the year prior on an industry supplied ration but that fawn died within 24 hours. This could have been from a lack of proper colostrum provided by the Doe.

Since both a buck and Doe are needed for a successful breeding producing fawns the new feed shows that it works both ways. Current status that is particular is that this buck (Red1) has now bred

and produced fawns out of 3 different does where as all 3 Does have now been deceased with CWD onboard after being exposed in a 3 - 41/2 year time span.

Tackling reproduction deficiency in the Peck Farm deer exposed to CWD proved successful as pregnancy was verified in both Yellow 2 and Purple 1 this year. This is significant because it demonstrates that though Yellow 2 was verified to be rectal positive for CWD her hormone pathway for reproduction was not degraded enough to block carrying a fawn. This provides an important timeline of disease progression since she successfully gave birth on July 24$^{th.}$. Though Yellow 2 was a positive rectal detect for CWD in April of the year, her health status seemed to be protected and intact to go the full term of her pregnancy. This can help establish a timeline that Yellow 2 had maintained her hormone function before her decline post fawning. Coincidently, the 1st to 2nd week in August is the same time of the year deer hormonally start the timely process to change over to their winter costs and shed their antler velvet, evidence of hormonal change. With Yellow 2 starting her decline at this same time of the year with a shortening day length demonstrates that her hormone system is now fully impaired. This is also the time of the year where the hormones provide the body with the important function for what is termed fat partitioning. This provides the deer the capacity to put on a winter coat, add body fat and ready reproductive status in both sexes of deer. This is also the same time of the season that has the highest bacterial organism load in the environment.

Yellow 2 was under continued stress by a lifetime of bacterial stress exposure, carrying full term / late fawning, contracting new environmental pathogenic bacteria organisms supporting mastitis / brain associated hormone interruption in her brain regions which lead to a condition defined as bacteremia.

Bacteremia is a common occurrence typically in postpartum dairy cattle. Further investigations are warranted to investigate the modes by and which type of bacteria colonizes the bloodstream in an animal population and the importance of bacteremia has on health and productivity of affected ruminant animals including reproduction success.

Bacteremia usually causes no symptoms, but sometimes bacteria can accumulate in certain tissues or organs and cause serious infections. Usually, bacteremia, particularly if it occurs during ordinary activities, does not cause infections because bacteria typically are present only in small numbers and are rapidly removed from the bloodstream by the immune system.

However, if bacteria are present long enough, not identified, and in large enough numbers, particularly in ruminants with a weakened immune system, bacteremia can lead to other infections and sometimes trigger a serious body wide response called sepsis.

Sepsis is described as systemic infection in which bacteria or their toxins get into the bloodstream and travel throughout the body, creating life-threatening illness. Bacteria or viruses in the blood can cause septicemia. Bacterial toxins in the blood cause a condition known as toxemia. Some types of toxin-forming bacteria enter the body via the GI tract, after damaging the gut lining and slipping through it, causing death. The ruminant goes into shock when internal organs are damaged and start shutting down. Any blood-borne infection may become life-threatening if the bacteria or their toxins continue to damage vital organs (10).

SUMMARY

Stress and the subsequent release of stress-related neuro-endocrine hormones adversely affect various aspects of ruminant health including food intake and rumination. Interaction within the animal may account for the deleterious effects of stress on ruminant health. One of those areas concern the ability of stress to modulate the microbial composition of the ruminant intestinal tract. The elucidation of such neuroendocrine–bacterial interactions in ruminants can lead to new ways to improve various aspects of animal health and production by creating a favorable environment that ultimately will foster a beneficial microbial composition that also retards the persistence of potential food-borne pathogens (11).

CWD is mostly know to many as the disease process with no known treatment that is deemed infectious and is either just managed as in the wild populations or managed / depopulated in the case of farmed cervids once detected. Across the globe to the dismay of many deer farmers, hunters and others with aspirations alike, the continued frustration with this CWD testing concept of just finding a prion in a continuum narrative is socially overwhelming by all stakeholders.

In the past 20 year history of CWD research to date, research only looks to develop or refine specific mechanisms of testing to provide either validated testing or early detection methods for the detection of the CWD prion. From various constructs like soil, feed sources, body parts or fluids like urine these testing platforms only seek to find a prion signature in determining a possible positive detection or non detection of a CWD prion in cervids. The Cervid farm industry and wildlife biologists alike would be better served by testing for bacterial pathogens that creates a negative health consequence in cervids, livestock and other wildlife species alike. With utilization of these testing methods for monitoring or early intervention as used in this study provides for early detection of root causes of disease potential or progression of pathogenic organisms.

This study was designed on a small deer farm where some control over the studied deer could look at finding what initiates this disease process. In determining the environmental factors that could lead to the starting point of a disease process would then look to review backwards the underpinning of this disease process in a more investigative approach for a root cause. As the process and environmental aspects of the farm location, feed and water inputs along with monitoring the deer’s health, a disease process took the lives of 3 deer over a 4 year period. We learned immensely from these dead deer as they provided some common aspects of a disease process from its starting origin with compelling timeframes of pathogenic organisms to the death of the deer.

In the data presented the study shows the continuance of some common environmental bacterial organisms some pathogenic / some not on the landscape. When these organisms are acquired or reside in wrong places within the deer’s body (blood, lungs, brain) they create a negative devastating effect of the deer’s immune / hormone system. This degrading condition depletes the health of the deer over time and without early detection or treatment leans towards ending their lives.

The study also set out to investigate the use of new cutting edge testing methods used for monitoring the deer’s health profile for pathogenic organisms exposed to the deer. Once identified and understood, then prospective interventions or proper treatments could be put into play in an effort to reduce or eliminate pathogenic organism’s negative health consequence.

The presented findings from the death of the 3 deer on the farm helped identifying specific bacterial organisms in wrong locations in the body. This is an important find as to help the Farmed Cervid industry and wildlife agencies alike to understand what disease organisms were present in these deer. In this new understanding will allow the start of developing additional management strategies could lead to the prevention of these bacterial organisms through proper nutrition, supportive health assessment, habitat stability and or early treatment intervention.

In Table 11. shows only 5 bacterial organisms in Yellow 2 that were found in other random farmed deer tested. Staphylococcus and Shigella- ecoli were found in various locations in Yellow 2 and other deer used as controls from multiple farms. Though organisms were found in control deer blood none of these organisms were found in any control deer fecals. This comparison of negative organisms from a deer dying with a life ending disease called CWD shows that there are some underlying health concerns detected in an early state if left unattended could diminish a deer's' health and wellbeing.

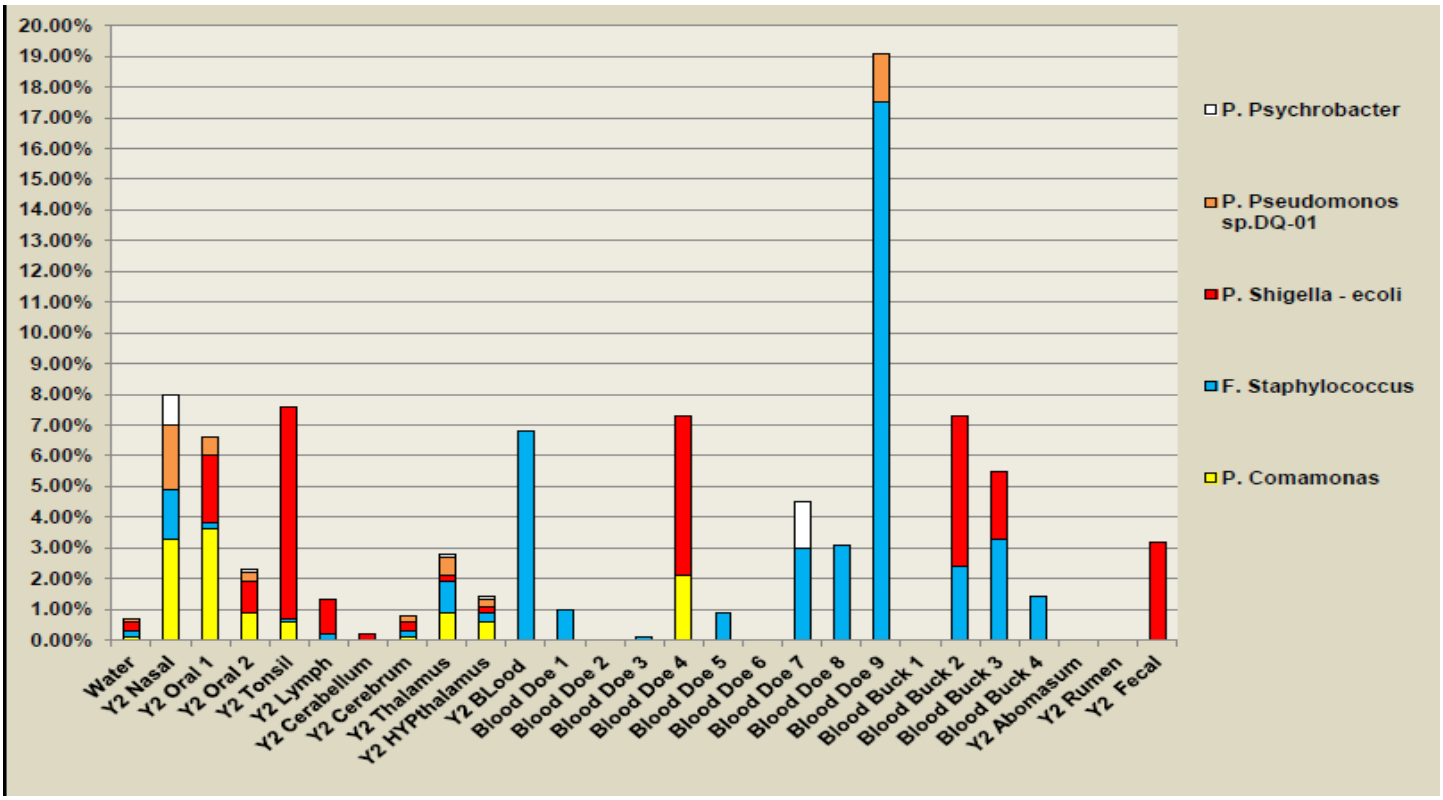


Table11. Some bacterial organisms found in Yellow 2 were also found in other random farm deer of both sexes in random samples from around the state. Early detection is important for maintaining health promotion in deer.

By developing a monitoring program for these or other pathogenic organisms in deer or the deer's environment (water, feed, habitat, other) will provide an extra layer of bio - security so these organisms don't create a negative stressful condition over time in deer. Continued negative stress from multiple environmental factors will end up compromising the immune system of deer at risk creating a negative health consequence.
This in turn reduces the deer's capability to stave off the onslaught of bacteria overload including invasion of the brain centers by bacterial toxic elements disrupting the body's hormone control systems. This leads to the cascading events of further bodily dysfunction including muscle and fat deterioration of the deer's body as a wasting condition. This bacteria overload in livestock including

deer is termed bacteremia. If not monitored for or detected early for proper treatment interventions will in time lead to the deer's death.

Continued follow up will be conducted on these deer as we enter year 5 of this study post exposure of these deer to the 1rst CWD death on this farm in January 2016.

You the member, hold the key in supporting this continued research so your input is highly valued.

Contact me or your Industry Leaders to have a conversation supporting CWD research.

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