# Peck Farm Phase 5 Report

This Phase 5 Research report will utilize past research generated in a continued effort to build upon the understanding of how negatively associated environmental organisms, when exposed to deer / elk result in the decline of the deer's immunity. By identifying these negative organisms, along with variability of geographic environmental exposures, we can continue to understand the risk factors for disease associated health conditions in deer / elk alike. Negative associated disease producing organisms, if left unchecked in practice, will lead to development of either an acute or chronic condition negatively impacting your deer / elk health and consequently your farms bottom line. In Phase 4.5 we identified some of these organisms for which are treatable with the help of your farm veterinarian for proper vaccine use. Other organisms identified were considered more antibiotic resistant and pose a greater health concern when left unchecked. Once known antibiotic resistant organisms or other pathogens gain a foot hold in deer / elk, they will lead to a further diminished health consequence now known to be a leading causal pathway in the development of the neurodegenerative disease we have come to know as Chronic Wasting Disease (CWD). Historically the disease causing organisms, identified through this research, have never been reported in cases of CWD positive detected deer / elk upon death on the farm or in the wild. Phase 5 continues to monitor the deer and elk on a CWD quarantined farm in an effort to provide critical information for the Cervid farm industry and wildlife personnel alike. In continuing to understand the environmental / disease associated pathways, it will help provide a pathway on how environmental organisms can / should be controlled in the survival in deer / elk both on the farm ad in the wild alike.

Background

The first index CWD case on this farm was in early January 2016. Purple 1 (last CWD + doe) was rectally tested in the spring of 2016, 2017, 2018 and 2019 testing as non-detect by IHC methods by NVSL. From the start of this study farm records show Purple 1 (doe) had not produced a fawn in the spring of 2015, 2016, 2017 or 2018. Since prior rations were used on each of the 3 farms in this study helped generate baseline findings for each these individualized farms. In the fall of 2018 an improved ration was designed and fed on the quarantined farm as well as both control farms in this study. Switching to a common ration was a way to provide a more complete picture of dietary similarities on 3 different farms in 3 different geographical locations under different health status' (CWD exposed / non-CWD exposed) of deer / elk. This new ration was designed to re-establish reproduction through improved health promotion via inflammation reduction due to negatively associated environmental organisms considered to diminish deer / elk health status. All deer on each farm started this new ration in the fall of 2018 prior to conception in the fall breeding season. Purple 1 delivered a buck fawn (Purple 2) on August 15, 2019 her first known fawn since the start of this study in spring 2016. By maintaining all deer on this ration Purple 1 subsequently produced a second buck fawn (Purple 3) on July 1,

2020 (45 days sooner vs. 2019 birth date) for which both buck fawns are still alive as of this report 8/2021.

Purple 1 was found deceased on November 19, 2020 and samples were collected for testing. Phase 4.5 also collected like control deer samples to compare like samples in the first comparison of a wild deer harvested from the CWD endemic zone within 1 mile of the quarantined farm along with other non – CWD control samples from a deer harvested in Northern Wisconsin during the 2020 hunting season.

See Phase 1 through Phase 4.5 for full background information.

Phase 5 Samples

In Phase 5 we will continue to monitor these deer and elk and collect saliva, blood and fecal from 3 bucks and 1 elk on the quarantined farm as well as from 1 buck and 1 elk from separate control farms. We also collected spring water samples to compare other water samples collected for identification of like bacteria organisms residing in water to animal transfer moving forward.

In this continuing effort to support the quarantine farm management, identifying and reducing or eliminating bacterial sources from the farm would provide for a higher level of bio-security. Improving surveillance and identifying actions to be taken, are needed to reduce bacterial burden loads in deer / elk. Other bio-security health measures include the consideration of the origin of the products brought to the farm and include transferred deer / elk from other geographical locations. By continuing to develop your farms best management practice, through the use of an effective vaccine program, would reduce the risk of certain bacteria with capabilities to produce pore forming toxins. The new information provided in this research will look to continue providing the farmer with tools to improve health management of their deer / elk.

**Results**

Well water collected from farm sources in prior research showed variability of negative associated bacterial organisms on a seasonal basis. Seasonal variability in these non potable water sources revealed bacterial organisms with a greater bacterial load in the fall vs. the spring. This is due in part that most private wells relying on these waters sources for drinking water are considered non-potable water sources. Non potable water can harbor higher bacterial loads without proper monitoring or sanitation practices on a routine basis. The review for water on your farm should include all water distribution systems such as piping and hoses alike. For additional comparison, water was collected from other local and regional sources from farm, wild and domestic municipal water sources. Two local sources water in close proximity to the quarantined farm (Artisanal Pond, Artisanal pipe) are used alike by local wildlife and humans (drinking, swimming etc…). Water Samples were also collected from a municipal water source that would be considered safe potable water for human consumption.

In table 1, the water bacterial organisms identified were compared to each other as a total number of bacteria identified and total concentration (higher number = concentration) of all bacteria. We then compared the concentration of the top 20 bacteria as compared to the total bacterial load in each water source.

| Water Tested | Q Farm Old | Q Farm New | Artisanal Pond | Artisanal Pipe | C Farm | House |
|---|---|---|---|---|---|---|
| Total # Bacteria Identified | 168 | 515 | 201 | 624 | 696 | 226 |
| Total Concentration of all Bacteria | 56,352 | 38,716 | 13,421 | 52,413 | 57,865 | 12,010 |
| Concentration of Top 20 Bacteria | 50,365 | 261 | 1,288 | 578 | 4,327 | 4,945 |
| Percentage Concentration in Top 20 | 89.4 | 0.007 | 9.5 | 1.1 | 7.5 | 41 |

**Table 1. Water sourced bacteria identified and compared. Quarantined farm artisan well (Q Farm Old), quarantine farm new well sourced water (Q Farm New), local artisanal well supplying a local pond 2 miles from quarantined farm (Artisanal Pond), well pipe from rock outcropping used as local drinking water source (Artisanal Pipe), control farm well water supply (C Farm) in non- CWD area and large city domestic house water (Home).**

The total bacterial count was higher in the Q Farm New water vs. the lower total bacterial count in the Q Farm Old water source, but is not of a current concern. What is of concern is the top 20 bacteria identified in the Q Farm Old water source made up 89.4% of the total bacteria concentration that were being consumed by the deer / elk on the quarantined farm. The Q Farm New water source shows only 0.007% of the top 20 bacterial organisms identified in the water source. Now this doesn't mean we are out of the woods regarding better water. The new water source is still deemed to be a non-potable water source that is delivered on the farm.

Next we compared the top 20 bacteria identified from all water sources (Table 2) tested in this part of the scientific review. The top organism identified (Thiothrix) in the Q Farm Old water shows its presence at a higher rate vs. the fall water testing (25,459 vs.4) from Phase 4.5 , Table 2. This organism was identified in the old quarantined farm water source it was not identified in the Q Farm New water source. Acintobactor was identified at a higher rate in the Q Farm Old water source but was identified at a lower concentration in the Q Farm New and other public and private water sources including the municipal water source reviewed. Pseudomonas was also identified in all water sources reviewed with the exception of the municipal water source.

With the Q Farm New water source not showing the negative associated bacteria as associated with the Q Farm Old water source, we reviewed the top 20 bacterial organisms of the newer water source on the quarantined farm (Table 3). We identified a greater load of different Pseudomonas bacteria in the Q Farm New water source compared to the Q Farm old water source. Pseudomonas and other bacterial organisms such as Staphylococcus, Streptococcus and Fusobacterium were seen in all other water sources reviewed.

| Water Bacteria Load Comparison | Q Farm Old | Q Farm New | Artisanal Pond | Artisanal Pipe | C Farm | House |
|---|---|---|---|---|---|---|
| Proteobacteria; Thiothrix | 25,459 | 0 | 0 | 0 | 0 | 44 |
| Proteobacteria; Desulfovibrio | 4,739 | 0 | 0 | 0 | 0 | 8 |
| Proteobacteria; Sideroxydans | 4,625 | 0 | 0 | 0 | 2,566 | 69 |
| Firmicutes; Clostridia; Desulfosporosinus | 2,737 | 0 | 339 | 0 | 161 | 25 |
| Actinobacteria; Coriobacteriaceae | 2,114 | 0 | 0 | 0 | 0 | 34 |
| Proteobacteria; Ferriphaselus | 1,518 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Methylobacterium | 1,162 | 0 | 0 | 0 | 0 | 4,580 |
| Bacteroidetes; Bacteroidetes vadinHA17 | 1,148 | 0 | 0 | 0 | 0 | 17 |
| Proteobacteria; Desulfomonile | 1,099 | 0 | 0 | 0 | 0 | 12 |
| Euryarchaeota; Methanobacteriaceae | 1,001 | 0 | 9 | 0 | 66 | 14 |
| Proteobacteria; Acinetobacter | 929 | 65 | 65 | 67 | 56 | 28 |
| Proteobacteria; Thiobacillus | 700 | 0 | 391 | 0 | 1,465 | 0 |
| Proteobacteria; Rhodopseudomonas | 511 | 0 | 0 | 0 | 0 | 0 |
| Chloroflexi; KD4-96 | 507 | 0 | 0 | 0 | 0 | 0 |
| Actinobacteria; Microbacterium | 506 | 32 | 0 | 229 | 0 | 46 |
| Proteobacteria; Stenotrophomonas | 410 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Paracoccus | 320 | 31 | 18 | 0 | 0 | 68 |
| Proteobacteria; Pararhizobium-Rhizobium | 310 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Pseudomonas | 306 | 164 | 466 | 282 | 53 | 0 |
| Actinobacteria; Sanguibacter | 304 | 0 | 0 | 0 | 0 | 0 |

**Table 2. Top 20 water bacteria organisms identified from the contaminated well as compared (Q Farm Old vs. Q Farm New) to a new well water source on quarantined farm as compared to other water sources tested.**

| New Water Bacteria Load Comparison | Q Farm Old | Q Farm New | Artisanal Pond | Artisanal Pipe | C Farm | House |
|---|---|---|---|---|---|---|
| Proteobacteria; Aquabacterium | 0 | 8,678 | 0 | 0 | 0 | 0 |
| Proteobacteria; Burkholderiaceae | 0 | 7,808 | 0 | 141 | 0 | 126 |
| Proteobacteria; Pseudomonas | 150 | 1,765 | 566 | 491 | 650 | 15 |
| Actinobacteria; Kocuria ; Ambiguous_taxa | 0 | 1,528 | 0 | 57 | 0 | 0 |
| Proteobacteria; Sphingopyxis | 33 | 657 | 31 | 231 | 28 | 87 |
| Proteobacteria; Acetobacteraceae | 0 | 630 | 0 | 0 | 0 | 0 |
| Firmicutes; Staphylococcus | 44 | 435 | 156 | 240 | 350 | 166 |
| Proteobacteria; KCM-B-112 | 0 | 409 | 0 | 0 | 0 | 0 |
| Proteobacteria; Sphingomonas | 5 | 382 | 6 | 7 | 26 | 8 |
| Firmicutes; Streptococcus | 0 | 499 | 69 | 1,213 | 210 | 36 |
| Actinobacteria; Mobilicoccus | 0 | 308 | 0 | 0 | 0 | 0 |
| Verrucomicrobia; Prosthecobacter | 0 | 294 | 0 | 0 | 0 | 0 |
| Proteobacteria; Bradyrhizobium | 32 | 281 | 0 | 76 | 81 | 0 |
| Proteobacteria; Noviherbaspirillum | 0 | 272 | 0 | 0 | 0 | 0 |
| Proteobacteria; Massilia | 0 | 254 | 62 | 0 | 219 | 48 |
| Proteobacteria; Acetobacteraceae | 0 | 252 | 0 | 0 | 0 | 0 |
| Proteobacteria; Skermanella | 0 | 225 | 6 | 0 | 11 | 0 |
| Bacteroidetes; Porphyromonas | 0 | 207 | 24 | 16 | 309 | 5 |
| Fusobacteria; Fusobacterium necrophorum | 0 | 192 | 32 | 21 | 140 | 0 |
| Actinobacteria; Arthrobacter | 0 | 180 | 0 | 0 | 0 | 0 |

**Table 3. Top 20 water bacteria organisms identified from a new well water source as compared (Q Farm New vs. Q Farm Old) to a contaminated well on the quarantined farm as compared to other water sources tested.**

Some good news observed was what was absent from the Q Farm New water source were the majority of past negative associated organisms from the Q Farm Old water source.

Since the quarantined farm had the most negative associated bacteria identified we compared the top 20 water bacteria found in water to the saliva samples collected on the quarantined and control farms (Table 4). Acintobacter was the only organism that was consistent in the saliva of deer / elk tested across all farms that were directly tied to their respective farm water source. Two older deer on the quarantine farm ( Q Buck 1 / 2) show a higher count than the younger deer ( Q Buck 3 ) on the quarantine farm and control farm (Control Buck) deer with lower saliva values for acintobacter. The elk on the quarantined farm (Q Elk) had a lower count than the count found in the control elk (Control elk). This observation would suggest that even at a low amount in well water this negative associated bacterium (acintobacter) could get a foothold as a time / age / exposure element that could lead to future health consequences to deer / elk when exposed to increased stress conditions. The concern is this organism was also identified in past brain sections of CWD+ deer.

| Top 20 Water Bacteria Load - Saliva | Q Farm Old | Q Farm New | C Farm | Q Buck 1 | Q Buck 2 | Q Buck 3 | Control Buck | Q Elk | Control Elk |
|---|---|---|---|---|---|---|---|---|---|
| Proteobacteria; Thiothrix | 25,459 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Desulfovibrio sp. | 4,739 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Sideroxydans | 4,625 | 0 | 2,566 | 0 | 0 | 0 | 0 | 0 | 0 |
| Firmicutes; Clostridia; Desulfosporosinus | 2,737 | 0 | 161 | 0 | 0 | 0 | 0 | 0 | 0 |
| Actinobacteria; Coriobacteriaceae | 2,114 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Ferriphaselus | 1,518 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Methylobacterium | 1,162 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bacteroidetes; Bacteroidetes vadinHA17 | 1,148 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Desulfomonile | 1,099 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Euryarchaeota; Methanobacteriaceae | 1,001 | 0 | 66 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Acinetobacter | 929 | 65 | 56 | 4,194 | 4,137 | 120 | 292 | 1,200 | 19,909 |
| Proteobacteria; Thiobacillus | 700 | 0 | 1,465 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Rhodopseudomonas | 511 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chloroflexi; KD4-96 | 507 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Actinobacteria; Microbacterium | 506 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 18 |
| Proteobacteria; Stenotrophomonas | 410 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Paracoccus | 320 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Pararhizobium-Rhizobium | 310 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Pseudomonas | 306 | 164 | 53 | 0 | 0 | 0 | 0 | 0 | 0 |
| Actinobacteria; Sanguibacter | 304 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 4. Top 20 water bacteria organisms identified from Q Farm Old as compared to saliva bacteria identified from both deer and elk tested.**

Since the deer and elk on the quarantined farm had fewer identified saliva bacteria, as compared to the top 20 water bacteria counts, I used the oldest exposed deer (Q Buck 1) on the quarantined farm to compare his saliva bacteria to other deer and elk in this portion of the study.

The 3 most concentrated bacterial taxa (Table 5) from the saliva of Q Buck 1 were not connected to any of the respective farm water sources tested. Bacteria organisms that were identified in deer / elk saliva such as Streptococcus, Mannheimia and Pasteurellaceae were not identified from their respective water sources on the farm.

| Top 20 Bacteria Load Q Buck 1 - Saliva | Q Farm Old | Q Farm New | C Farm | Q Buck 1 | Q Buck 2 | Q Buck 3 | Control Buck | Q Elk | Control Elk |
|---|---|---|---|---|---|---|---|---|---|
| Proteobacteria; Bibersteinia | 0 | 0 | 0 | 26,153 | 12,632 | 2,379 | 22,944 | 1,260 | 651 |
| Proteobacteria; Alysiella | 0 | 0 | 0 | 9,306 | 2,369 | 132 | 219 | 1,012 | 16,163 |
| Firmicutes; Streptococcus | 0 | 0 | 0 | 6,100 | 238 | 690 | 6,308 | 7,047 | 232 |
| Proteobacteria; Acinetobacter | 929 | 65 | 56 | 4,194 | 4,137 | 120 | 292 | 1,200 | 19,909 |
| Firmicutes; methanogenic archaeon | 0 | 0 | 0 | 4,110 | 2,677 | 1,965 | 5,623 | 0 | 0 |
| Bacteroidetes; Chryseobacterium | 0 | 0 | 0 | 1,936 | 128 | 0 | 0 | 0 | 270 |
| Proteobacteria; Mannheimia | 0 | 0 | 0 | 1,924 | 42 | 2,379 | 383 | 0 | 0 |
| Proteobacteria; Pasteurellaceae | 0 | 0 | 0 | 1,138 | 240 | 50 | 523 | 5,354 | 0 |
| Bacteroidetes; Bacteroidales; F082 | 0 | 0 | 0 | 1,079 | 1,198 | 427 | 0 | 0 | 0 |
| Bacteroidetes; Weeksellaceae | 0 | 0 | 0 | 770 | 161 | 37 | 66 | 0 | 68 |
| Bacteroidetes; Bergeyella | 0 | 0 | 0 | 713 | 576 | 0 | 134 | 0 | 0 |
| Bacteroidetes; Bergeyella | 0 | 0 | 0 | 671 | 86 | 24 | 43 | 0 | 1,872 |
| Fusobacteria; Fusobacterium necrophorum | 0 | 192 | 140 | 548 | 0 | 0 | 0 | 123 | 7 |
| Bacteroidetes; Prevotellaceae UCG-003 | 0 | 0 | 0 | 510 | 693 | 0 | 0 | 0 | 0 |
| Fusobacteria; Caviibacter | 0 | 73 | 41 | 470 | 9 | 0 | 262 | 6 | 0 |
| Bacteroidetes; Prevotella 1 | 0 | 0 | 0 | 360 | 264 | 279 | 0 | 0 | 0 |
| Bacteroidetes; Rikenellaceae RC9 gut | 0 | 0 | 0 | 346 | 749 | 276 | 0 | 0 | 0 |
| Firmicutes; Ruminococcaceae NK4A214 | 0 | 0 | 0 | 330 | 109 | 240 | 0 | 0 | 0 |
| Bacteroidetes; Prevotellaceae UCG-003 | 0 | 0 | 0 | 309 | 573 | 0 | 0 | 0 | 0 |
| Bacteroidetes; Porphyromonas | 0 | 207 | 309 | 307 | 0 | 0 | 0 | 10 | 0 |

**Table 5. Comparing the 20 bacterial organisms found in saliva of Q Buck 1 (longest exposed deer to CWD) as compared to respective well water and saliva of other quarantined / control deer and elk.**

Next, we reviewed the top 20 water bacteria from the quarantine farms old and new water supply in comparison for any connection to the deer / elk blood tested (Table 6). There were no water supplied bacteria identified from the top 20 bacteria organisms in well water that were associated with the deer / elk blood reviewed. This is a positive note since in the past the deer had water associated bacteria (shigella-ecoli) found in the bloods of the quarantined deer.

As we compared deer and elk for the saliva, we used the same comparison for the blood bacterial content in comparison to the oldest deer on the quarantined farm to other deer / elk (Q Buck 1, Table 7).

| Top 20 Water Bacteria Load - Blood | Q Farm Old | Q Farm New | C Farm | Q Buck 1 | Q Buck 2 | Q Buck 3 | Control Buck | Q Elk | Control Elk |
|---|---|---|---|---|---|---|---|---|---|
| Proteobacteria; Thiothrix | 25,459 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Desulfovibrio sp. | 4,739 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Sideroxydans | 4,625 | 0 | 2,566 | 0 | 0 | 0 | 0 | 0 | 0 |
| Firmicutes; Clostridia; Desulfosporosinus | 2,737 | 0 | 161 | 0 | 0 | 0 | 0 | 0 | 0 |
| Actinobacteria; Coriobacteriaceae | 2,114 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Ferriphaselus | 1,518 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Methylobacterium | 1,162 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bacteroidetes; Bacteroidetes vadinHA17 | 1,148 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Desulfomonile | 1,099 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Euryarchaeota; Methanobacteriaceae | 1,001 | 0 | 66 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Acinetobacter | 929 | 65 | 56 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Thiobacillus | 700 | 0 | 1,465 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Rhodopseudomonas | 511 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chloroflexi; KD4-96 | 507 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Actinobacteria; Microbacterium | 506 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Stenotrophomonas | 410 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Paracoccus | 320 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Pararhizobium-Rhizobium | 310 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Pseudomonas | 306 | 164 | 53 | 0 | 0 | 0 | 0 | 0 | 0 |
| Actinobacteria; Sanguibacter | 304 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 6. Of the top 20 identified water bacteria found in the quarantine farm water supply none were found in quarantined or control deer and elk sampled.**

The leading blood bacteria identified in Q Buck 1 was also found in the water supply and bloods of respective deer / elk tested. Though this water supplied bacteria didn't make the top 20 bacteria concentration list this review seeks to identify all aspects of potential contamination.

| Total Bacteria Load Q Buck 1 - Blood | Q Farm Old | Q Farm New | C Farm | Q Buck 1 | Q Buck 2 | Q Buck 3 | Control Buck | Q Elk | Control Elk |
|---|---|---|---|---|---|---|---|---|---|
| Bacteroidetes; Pedobacter | 42 | 120 | 87 | 316 | 348 | 328 | 260 | 317 | 352 |
| Tenericutes; Mycoplasma haemocervae | 0 | 0 | 0 | 280 | 257 | 5,276 | 165 | 25 | 0 |
| Tenericutes; Mycoplasma erythrocervae | 0 | 0 | 0 | 262 | 0 | 0 | 0 | 0 | 0 |
| Firmicutes; Clostridia; Christensenellaceae R-7 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 |
| Bacteroidetes; Bacteroides | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| Firmicutes; Clostridia; Clostridiales vadinBB60 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| Tenericutes; Anaeroplasma;Ambiguous_taxa | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 5 | 0 |
| Euryarchaeota; Methanobrevibacter | 0 | 24 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |

**Table 7. Comparing all bacterial organisms identified in blood of Q Buck 1 (longest exposed deer to CWD) as compared to other quarantined / control deer and elk.**

Other bacterial organisms identified at lower counts in deer and elk not associated with the farm water supply were 2 strains of Mycoplasma.

In this review, when we compared bacteria found in the farm water supply, we found little association of the deer/elk with the water supply. In fact the deer/elk blood shows low blood bacteria when considering the farm water supply (Table 8). This is quite a change from past blood testing demonstrating bacterial contamination of deer / elk on the quarantine farm from (Phase 1 through Phase 4.5).

| Water Associated Bacteria Load - Blood | Q Farm Old | Q Farm New | C Farm | Q Buck 1 | Q Buck 2 | Q Buck 3 | Control Buck | Q Elk | Control Elk |
|---|---|---|---|---|---|---|---|---|---|
| Bacteroidetes; Pedobacter | 42 | 120 | 87 | 316 | 348 | 328 | 260 | 317 | 352 |
| Tenericutes; Mycoplasma haemocervae | 0 | 0 | 0 | 280 | 257 | 5,451 | 165 | 25 | 0 |
| Tenericutes; Mycoplasma erythrocervae | 0 | 0 | 0 | 262 | 0 | 0 | 30 | 0 | 0 |
| Bacteroidetes; Muribaculaceae | 0 | 0 | 0 | 0 | 3 | 2 | 3 | 0 | 0 |
| Actinobacteria; Brachybacterium | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| Firmicutes; Ruminiclostridium 9 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| Patescibacteria; Candidatus Saccharimonas | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| Firmicutes; Staphylococcus | 44 | 435 | 350 | 0 | 2 | 2 | 0 | 0 | 0 |
| Bacteroidetes; Odoribacter | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Firmicutes; Clostridiales vadinBB60 group | 0 | 0 | 0 | 4 | 0 | 4 | 0 | 0 | 0 |
| Firmicutes; Clostridia; Lachnospiraceae | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Bacteroidetes; Bacteroides | 0 | 0 | 0 | 4 | 9 | 0 | 0 | 3 | 0 |
| Firmicutes; Clostridia; Family XI; Peptoniphilus | 0 | 10 | 3 | 0 | 3 | 0 | 0 | 0 | 0 |
| Firmicutes; Clostridia; Anaerotruncus | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Firmicutes; Clostridia; Christensenellaceae R-7 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 |
| Tenericutes; Anaeroplasma | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 5 | 0 |
| Euryarchaeota; Methanobrevibacter | 0 | 24 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |

**Table 8. Comparing all deer / elk blood bacteria identified that were common to any water sources on respective farms.**

The only blood bacteria in common with both elk was Pedobacter and was associated with the water source and deer alike (Table 8). All other blood bacteria identified were unique to each elk on each respective farm. Though Pseudomonas was found in the water source of both the quarantine and control farm they were not identified in the respective elk's blood. Mycoplasma haemoplasma was found only in the quarantined elks blood whereas mycoplasma haemobos, erythrocerve and wenonii were identified and unique to the control elk tested. None of the mycoplasma identified were from any farm well water sources.

Next we reviewed the water supply for the quarantined and control farm for the Elk. Reviewing for differences of water born bacteria between bloods of the elk shows a distinct difference of blood profile from 2 different farm water sources. The only common organism found in Elk blood was the water sourced Pedobacter. This could be important as when Elk are moved from farm to farm in commerce that they have the potential to pick up a new bacterial load not found in their respective farm water supply (Table 9).

| Bacteria Associated Q Elk 1 Load - Blood | Q Farm Old | Q Farm New | C Farm | Q Elk | Control Elk |
|---|---|---|---|---|---|
| Bacteroidetes; Pedobacter | 42 | 120 | 87 | 317 | 352 |
| Actinobacteria; Arthrobacter | 0 | 257 | 9 | 80 | 0 |
| Tenericutes; Mycoplasma haemocervae | 0 | 0 | 0 | 25 | 0 |
| Firmicutes; Bacillus | 0 | 99 | 19 | 16 | 0 |
| Firmicutes; Planococcaceae | 0 | 0 | 0 | 10 | 0 |
| Firmicutes; Clostridia; Eubacterium | 0 | 7 | 0 | 10 | 0 |
| Tenericutes; Anaeroplasma | 0 | 0 | 0 | 5 | 0 |
| Firmicutes; Clostridia; Lachnospiraceae NK3A20 | 0 | 4 | 0 | 5 | 0 |
| Proteobacteria; Pseudomonas | 0 | 1,251 | 468 | 5 | 0 |
| Bacteroidetes; Muribaculum | 0 | 0 | 0 | 4 | 0 |
| Firmicutes; Clostridia; Lachnospiraceae | 0 | 0 | 0 | 4 | 0 |
| Bacteroidetes; Bacteroides | 0 | 0 | 0 | 3 | 0 |
| Firmicutes; Clostridia; Mogibacterium | 0 | 0 | 0 | 3 | 0 |
| Firmicutes; Clostridia;Ruminococcaceae UCG-005 | 0 | 0 | 0 | 3 | 0 |
| Tenericutes; Mycoplasma haemobos | 0 | 0 | 0 | 0 | 353 |
| Tenericutes; Mycoplasma erythrocervae | 0 | 0 | 0 | 0 | 131 |
| Tenericutes; Mycoplasma wenyonii | 0 | 0 | 0 | 0 | 93 |
| Bacteroidetes; Muribaculaceae | 0 | 0 | 0 | 0 | 8 |
| Bacteroidetes; Hymenobacter | 0 | 0 | 0 | 0 | 3 |
| Firmicutes; Lactobacillus murinus | 0 | 0 | 0 | 0 | 3 |
| Bacteroidetes; Alistipes | 0 | 0 | 0 | 0 | 2 |
| Rokubacteria; NC10; Rokubacteriales | 0 | 0 | 0 | 0 | 2 |

**Table 9. Comparisons of water source bacteria to Q Elk and control Elk Blood**

Next we reviewed the top 20 water bacteria identified in each of the farms water supply and compared them for any connection to the deer / elk fecal tested (Table 10). There were only a few low count bacterial organisms found relating to water supplied to the deer / elk fecal. This may show that these current comparisons can be an effective tool since past testing has demonstrated water supplied organisms in fecal material. Since we have a current cleaner water supply helps in minimizing negative associated bacteria traveling through deer / elk (Phase 4.5).

With this we then compared the fecal bacteria content in the oldest deer on the quarantined farm to other deer / elk (Q Buck 1, Table 11). Though there were some clostridia bacteria found in each of the water supplies on each farm, they were of a low count. In comparison of each respective fecal bacterium loads of deer / elk the deer on the quarantined farm show a higher count of like clostridia organisms than on the control farms. Though Clostridium Sensu Stricto 1

was identified in the Q farms water supply this spring but not from the Q farms fall water supply it

| Top 20 Water Bacteria Load - Fecal | Q Farm Old | Q Farm New | C Farm | Q Buck 1 | Q Buck 2 | Q Buck 3 | Control Buck | Q Elk 1 | Control Elk |
|---|---|---|---|---|---|---|---|---|---|
| Proteobacteria; Thiothrix | 25,459 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Desulfovibrio sp. | 4,739 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Sideroxydans | 4,625 | 0 | 2,566 | 0 | 0 | 0 | 0 | 0 | 0 |
| Firmicutes; Clostridia; Desulfosporosinus | 2,737 | 0 | 161 | 0 | 0 | 0 | 0 | 0 | 0 |
| Actinobacteria; Coriobacteriaceae | 2,114 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Ferriphaselus | 1,518 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Methylobacterium | 1,162 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bacteroidetes; Bacteroidetes vadinHA17 | 1,148 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Desulfomonile | 1,099 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Euryarchaeota; Methanobacteriaceae | 1,001 | 0 | 66 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Acinetobacter | 929 | 65 | 56 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Thiobacillus | 700 | 0 | 1,465 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Rhodopseudomonas | 511 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chloroflexi; KD4-96 | 507 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Actinobacteria; Microbacterium | 506 | 32 | 0 | 0 | 46 | 0 | 0 | 15 | 22 |
| Proteobacteria; Stenotrophomonas | 410 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Paracoccus | 320 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Pararhizobium-Rhizobium | 310 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proteobacteria; Pseudomonas | 306 | 164 | 53 | 0 | 0 | 0 | 0 | 0 | 0 |
| Actinobacteria; Sanguibacter | 304 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 10. Comparison of farm water bacteria identified in fecal of deer and elk.**

| Top 20 Bacteria Load Q Buck 1 - Fecal | Q Farm Old | Q Farm New | C Farm | Q Buck 1 | Q Buck 2 | Q Buck 3 | Control Buck | Q Elk 1 | Control Elk |
|---|---|---|---|---|---|---|---|---|---|
| Firmicutes; Clostridia; Lachnospiraceae NK3A20 | 0 | 4 | 0 | 9,906 | 3,352 | 1,281 | 1,205 | 828 | 366 |
| Euryarchaeota; Methanobrevibacter | 0 | 24 | 0 | 8,928 | 8,544 | 3,445 | 2,952 | 763 | 292 |
| Firmicutes; Clostridia; Eubacterium | 0 | 7 | 0 | 5,013 | 4,008 | 4,006 | 2,155 | 346 | 226 |
| Firmicutes; Clostridia; Christensenellaceae R-7 | 0 | 0 | 0 | 2,924 | 3,963 | 2,933 | 375 | 2,298 | 892 |
| Firmicutes; Clostridia; Peptostreptococcaceae | 0 | 13 | 0 | 2,620 | 2,044 | 1,975 | 1,225 | 2,994 | 537 |
| Firmicutes; Clostridia; Romboutsia | 0 | 57 | 18 | 2,262 | 1,695 | 1,147 | 403 | 2,388 | 323 |
| Firmicutes; Clostridia; Mogibacterium | 0 | 0 | 0 | 1,638 | 316 | 221 | 803 | 3,050 | 2,240 |
| Firmicutes; Clostridia; Ruminococcaceae UCG-002 | 0 | 0 | 0 | 1,424 | 1,404 | 394 | 28 | 276 | 198 |
| Firmicutes; Clostridia; Acetitomaculum | 0 | 0 | 0 | 1,313 | 1,768 | 678 | 963 | 101 | 0 |
| Firmicutes; Clostridia; Clostridium sensu stricto 1 | 0 | 20 | 12 | 1,291 | 45 | 0 | 0 | 0 | 18 |
| Actinobacteria; Olsenella | 0 | 0 | 0 | 1,213 | 406 | 138 | 171 | 163 | 71 |
| Firmicutes; Clostridia; Family XIII AD3011 | 0 | 0 | 0 | 1,171 | 1,220 | 436 | 74 | 1,233 | 341 |
| Bacteroidetes; Bacteroides | 0 | 0 | 0 | 893 | 707 | 2,622 | 17 | 511 | 912 |
| Firmicutes; Erysipelotrichia; Turicibacter | 0 | 0 | 0 | 835 | 377 | 275 | 1,278 | 2,034 | 248 |
| Euryarchaeota; Methanobrevibacter | 0 | 0 | 0 | 732 | 63 | 34 | 385 | 136 | 0 |
| Actinobacteria; Eggerthellaceae; DNF00809 | 0 | 0 | 0 | 585 | 51 | 0 | 0 | 19 | 88 |
| Firmicutes; Clostridia; Romboutsia | 0 | 0 | 0 | 477 | 483 | 714 | 190 | 474 | 42 |
| Firmicutes; Bacilli; Bacillus | 0 | 90 | 57 | 442 | 497 | 577 | 123 | 716 | 204 |
| Bacteroidetes; Rikenellaceae RC9 | 0 | 0 | 0 | 434 | 480 | 270 | 0 | 0 | 0 |
| Firmicutes; Turicibacter | 0 | 0 | 0 | 362 | 145 | 75 | 303 | 0 | 0 |

**Table 11. Comparison of bacteria load from Q Buck 1 to other deer and elk fecal**

was still identified at a higher concentration in the older buck fecal, as compared to other deer and elk. This is another clostridia species of interest and concern as it was also identified in the past from this quarantined farm tied to deceased deer that tested positive with CWD. There were many other bacterial organisms identified in deer and elk that were not associated with the farms water source from this spring's sampling. The lower negative bacterial count, along with the absence of other bacteria of concern, shows a promising profile of deer and elk tested in this spring's timeframe of the study. Rechecking the fall water supply would be warranted in review of seasonal fluctuations.

In comparison of the elks fecal profiles on the quarantined and control farm though there were similarities of fecal bacteria residing in both elk. Some bacteria were more concentrated for some organisms in the quarantined elk (Table 12) during the spring time review.

| Top 20 Bacteria Load Q Elk 1 - Fecal | Q Farm Old | Q Farm New | C Farm | Q Elk 1 | Control Elk |
|---|---|---|---|---|---|
| Euryarchaeota; Methanobrevibacter | 0 | 0 | 0 | 6,568 | 396 |
| Firmicutes; Clostridia; Christensenellaceae R-7 | 0 | 0 | 0 | 6,187 | 1,178 |
| Firmicutes; Clostridia; Ruminococcaceae UCG-005 | 0 | 7 | 0 | 3,594 | 5,485 |
| Firmicutes; Clostridia; Mogibacterium | 0 | 0 | 0 | 3,463 | 2,240 |
| Firmicutes; Clostridia; Peptostreptococcaceae | 0 | 13 | 0 | 2,994 | 537 |
| Firmicutes; Clostridia; Romboutsia | 0 | 57 | 18 | 2,861 | 365 |
| Firmicutes; Turicibacter | 0 | 0 | 0 | 2,034 | 248 |
| Firmicutes; Clostridia; Family XIII AD3011 | 0 | 0 | 0 | 1,692 | 341 |
| Firmicutes; Bacilli; Bacillus | 0 | 90 | 57 | 1,164 | 204 |
| Bacteroidetes; Rikenellaceae; Alistipes | 0 | 0 | 0 | 832 | 188 |
| Euryarchaeota; Methanobrevibacter | 0 | 24 | 0 | 763 | 292 |
| Firmicutes; Clostridia; Paeniclostridium | 0 | 0 | 0 | 609 | 0 |
| Euryarchaeota; Methanobrevibacter | 0 | 0 | 0 | 582 | 335 |
| Firmicutes; Clostridia; Ruminococcaceae UCG-014 | 0 | 0 | 0 | 561 | 0 |
| Firmicutes; Clostridia; Lachnospiraceae NK3A20 | 0 | 4 | 0 | 534 | 254 |
| Firmicutes; Clostridia; Clostridium sensu stricto 1 | 0 | 20 | 12 | 527 | 18 |
| Firmicutes; Clostridia; Ruminococcus 2 | 0 | 0 | 0 | 520 | 44 |
| Bacteroidetes; uncultured Bacteroides sp. | 0 | 0 | 0 | 511 | 912 |
| Verrucomicrobia; Akkermansia | 0 | 0 | 0 | 403 | 15 |
| Firmicutes; Clostridia; coprostanoligenes | 0 | 0 | 0 | 341 | 103 |

**Table 12. Bacteria as compared from Q Elk 1 to Control elk.**

## Discussion

This spring's collection of samples, from deer / elk on a farm held under quarantine for CWD, continues to add to our base knowledge of this disease process; the why / how deer / elk develop CWD. Using deer / elk samples from control farms in this study is crucial for demonstrating our findings from CWD exposed deer / elk in comparison to control samples.

To understand the disease process of animals under quarantine one must have control samples to compare apples / apples. This is important as we review how an animal's immune system functions properly.

The body's immunity is activated daily to modulate and correct acute inflammation processes. Problems arise when constant inflammation becomes a chronic inflammatory condition (10).

These conditions generally are characterized as a sub-acute inflammation which typically goes unnoticed. These sub-acute chronic inflammatory conditions, if left unchecked over a longer timeframe, could slowly be working against the immune system. Left over a period of time could lead to a diagnosis of amyloidosis. Amyloidosis is defined as a cellular protein miss-folding complex / process in the body, could be localized in a specific organ or tissue whereas and result in a cascading disease processes' that describes transformation in scrapies and CWD. (11)
We have previously identified negative bacterial organisms that are tied directly to development of amyloid B-sheet formation (1) and integration into the mammalians body complexes (2). We are providing a path forward in this study, in answering questions asked by our industry regarding the potential mitigation process, and demonstrating pathways of a disease process.

With this knowledge we will look to move forward in a potential recovery mode with the deer / elk in this study by implementing identified improvements to areas of farm health.

On the quarantined farm, we will provide a newer water supply for the deer / elk. Previous water testing showed seasonal variability (3) and we identified unhealthy, and possibly disease causing, bacteria delivered to the deer in their water. These organisms were confirmed to be present in multiple CWD positive deer tissues (3, 4, and 5) that are considered the causal actors in protein amyloidal B-sheet formation resulting in the detection of CWD in deer upon death.
The use of a newer water source provides for less bacterial exposure to deer / elk with continued testing to demonstrate a continued lower bacterial presence in the new farm water supply for the deer / elk. Continued monitoring for bacterial loads in water is warranted due to seasonal changes in water quality, especially considering non-potable water supply to any farm deer / elk or other livestock. Continuing to monitor well water will help detect potentially unhealthy organisms in both farmed and wild deer, located in the endemic region, when compared to control farmed or wild deer / elk samples tested from non- CWD affected areas in Wisconsin (5, ref.1 - 12).
The second area of improvement was in refinement to the feed provided to the deer / elk on the quarantined farm. Whether providing a pellet or textured feed, it is important to know what is considered appropriate for the deer / elk to maintain healthy growth for each age group in the farm production cycle. A consistent feeding program helps to maintain proper rumen function by keeping appropriate microbial loads in deer and elk. The ration provided on the quarantined farm is designed in a nutrient manner to support key enzymatic pathways that support proper protein folding complexes when challenged by certain bacterial with the capacity of producing toxins leading to B-sheet formation. Introduction of untested or off branded feed products of unknown nutritional composition were found to add to the stress burden of the production expectation of a healthy herd. The same is true when providing inferior forage products which have unbalanced nutritional values, have gone untested, and could introduce unknown risk factors when fed (6).

A third area of improvement / importance in any farm production is implementation of a vaccine protocol for your livestock (7, 8, and 9) in consultation with your farm veterinarian. This is important since geographically there are different areas of organism's reservoirs' in the wild and farm alike. In the beginning of this research effort it was noted that the deer / elk on the

quarantined farm had never been vaccinated. This provided an opportunity to review the original question of what caused CWD in deer and elk. Un-vaccinated deer / elk allowed for providing additional important information to unravel this common disease that afflicts the cervid farm community and evades wildlife professional's knowledge. These findings will help in dealing with CWD in the farming community along with wild deer / elk populations in multiple states. Improvements in farm management will help pave the way for any farmer support their herd(s) in a positive manner towards heard health.

## Summary

In previous research reports (3, 4, 5) we have continued to advance new knowledge to better understand this disease process called CWD. We have currently passed the 5 year mark of CWD exposure to deer / elk, from the first index case on this farm, in research with live animals under CWD quarantine conditions. In this time, the oldest buck has bred 5 different Does, now deceased and confirmed CWD positive. We are currently left with 3 whitetail bucks (6.5 years, 2.5 years and 1 year), and 1 bull elk (12 years) at 5 ½ years post CWD exposure. These animals will continue to provide important future pathways forward in the continuing understanding in the investigation into the root causes of CWD and mitigation efforts to date for the farm cervid industry.

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Review all research reports - contact your Board members re: continuing our CWD research**.**
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