Peck Farm Research Report Phase 6

This Phase 6 Research report is an update on the deer and elk that remain on a quarantined farm 6 years post detection of the first index case of CWD in whitetail deer. As we enter the 7th year of CWD exposure, on this premise, past analysis supports detrimental health effects of environmental bacterial organisms. These organisms create the process of pathogen-associated molecular patterns (PAMPs) leading to endogenous danger signals through the sensing of danger-associated molecular patterns (DAMPs) (1) when exposed to deer / elk. These bacterial processes, including secondary metabolites, and or pore forming toxins have the capacity to transform the a-helic protein (PrPc) to a mis-folded beta sheet protein (PrPsc) prion. These bacterial species and their survival processes, when exposed to cervids and left untreated, will eventually lead to a cervid developing Chronic Wasting Disease (CWD).

Phase 6

In Phase 6 the plan was to continue monitoring and support the health of the remaining 3 bucks and 1 bull elk on the quarantined farm. In addition health monitoring was continued for 1 buck and 1 bull elk from negative CWD control farms used in this study.

Results

In April, vaccinations were planned for the deer and elk. At that time it was noted that the older buck, Red1, had developed another jaw infection 1week before his 7th year of age. This was the 3rd infection in his lifetime. His first infection was small, along the lower jaw line, and resolved itself in July of 2020. No follow-up treatment was provided at that time. The second infection resurfaced later that December for which was more robust in size. The deer was anesthetized (dart tranquilized) and the abscess was drained, flushed and treated with appropriate antibiotics. The organisms identified from this infection were also found to be sourced from the farms drinking water supply during this same time period of infection onset. Unfortunately, this spring by the time we were able to attempt treatment for a jaw infection the buck was found deceased on May 4th, 2022. Regulatory samples were collected and submitted to NVSL for testing for CWD by IHC method. Other samples for testing follow up were unavailable due to the warmer weather and time from death. The buck was confirmed to be CWD positive in both lymph nodes and obex by IHC at NVSL.

Background Discussion

References published in previous phases of this research (2) provide a full pathway of what, how and where bacterial organisms are associated in the development of CWD in deer and elk. These bacterial organisms were only found on the quarantined farm or in the wild deer population in SW Wisconsin. They were not, however, found in deer and elk on control farms or the wild deer in this study from outside of the SW Wisconsin CWD Endemic zone. Though this research is to support farm management of deer and elk it can also be helpful for wildlife professionals alike.

For farm management practices, it is always best to develop a bio-security plan for each farm. However, a one size fits all approach could be challenging due to geographical location. In addition varying environmental conditions such as bacterial loads must be considered. Geographic location may dictate bacterial loads and should be identified early. Prevention and proper treatment protocols will be dependent on that information and can minimize herd exposure. This can be done with the help of the farm or wildlife veterinarian in proper bacterial identification to support selection of vaccine(s), antibiotics, or diagnostics when needed. Preventative healthcare is a key element in any herd Bio-security Action Plan as some bacteria may continue to become more antibiotic resistant during treatment and pose a greater health concern in farmed or wild deer and elk alike. Being vigilant and continually updating and following a preventative health plan prevents continued bacterial exposure in deer or elk.

Feed, forage or water could be sources of unwanted bacterial exposures in deer and elk. Bacteria could then gain a foot hold and potentially result in negative health consequences. A continued diminished health consequence, in turn, has been identified to reveal a bacterial pathway in a cervid in the development of a neurodegenerative disease consequence known as Chronic Wasting Disease (CWD). (3)

Historically, most original research investigations looked at how a prion disease (CWD) is infectious by scientific dosing methods or by how the neurodegenerative effects were expressed in various animal models that ultimately lead to a prion transfer detection or death endpoint. This method has only provided different methodologies of developing different testing platforms for early detection or of a disease onset. Detection methodologies using various sourced passaged prions were either from live or subsequent dead animals (mice, hamsters,) including cervids. This seems to be the only way forward without the complete understanding and the underpinning of a biological disease process in itself.
In one research review of farmed WTD it is noted they are highly susceptible to systemic bacterial infections. Commonly cultured microorganisms from the gastrointestinal tract include *E. coli* and *Clostridium* spp., Bronchopneumonia as a common cause of death in farmed WTD. Bacteria commonly isolated from pneumatic lungs include *Arcanobacterium pyogenes, Fusobacterium necrophorum, Escherichia coli, and Mannheimia haemolytica*.

Mortality due to bacterial infection is particularly high in fawns under one year of age since they are immunologically naïve and are born during the summer when climatic conditions favor bacterial growth. These higher mortality rates may also be associated with a variety of hematologic and immunologic factors.

Many diseases affect both farmed and wild WTD in Florida and present major challenges to this relatively new and growing farm industry nationwide. Little work has been done to characterize the epidemiology of these diseases in wild deer and how adopting a traditional game species into a novel livestock species is affecting these disease dynamics. This domestication alters the life history traits of host, vector, pathogen, and therefore further characterization of the epidemiology of this novel system is necessary to make informed, sustainable management decisions. (4). Though this research focused on bacteria there were no attempts to review the association with deer developing CWD because prior thought of bacteria was deemed not to be involved.

Initial research (20+ years ago) provided a small research review of a few selected bacteria species as a potential causal agent to CWD but was determined that there was no compelling connection to bacteria leading to deer or elk developing CWD.

This initial research determination left an industry and science with a notion that bacteria were not a causal agent of CWD. This resulted in 20 + years of scientific testing development for earlier detection kits but there still was no research being conducted in relation to the initial disease development in cervids. The only other un-answered hypothesis is that CWD could be just a spontaneous development in cervids.

In reviewing some of the initial bacterial research, one initial prion experiment in 2010 noted that although the model being used was supported by rapidly growing experimental data, unequivocal proof has been elusive that lipids are non-obligatory for prion protein conversion to the infectious form. (5) But in 2016, updated research demonstrated that many bacterial pathogens have evolved the ability to interfere with host cell organelles. Noting the importance of cellular functions were compartmentalized in these organelles, such as DNA maintenance and gene expression in the nucleus, sorting of newly synthesized proteins and lipids in the ER and in the Golgi or bioenergetics and programmed cell death in mitochondria. Targeting of these organelles allows bacteria to manipulate key functions of the host cell in order to promote infection. One example of bacteria function like that of extra-cellular Listeria, via secretion of the pore -forming toxin listeriolysin O, alters lysosomal integrity in epithelial cells but not in macrophages (6).

Another bacterial research review investigated lipids as a potential facilitating factor because GPI-anchored PrPC is in the vicinity of lipid membranes and the interfacial lipid bilayer region strongly influences protein structure. Researchers were encouraged by the findings that lipid interaction converts recPrP to a PrPSc-like form, so they applied protein misfolding cyclic amplification (PMCA) to study recPrP conversion in the presence of both lipid and RNA. It was concluded that a requirement of lipid is in accordance with previous reports of higher prion infectivity in lipid membrane associated PrPSc. Notably, the purified GPI-anchored PrPC, which was used to produce infectious prion de novo, contained stoichiometric amounts of co-purified lipids, supporting a general role of lipid in PrP conversion to PrPSc.(7)

These bacterial process described (6, 7) further corroborates our current findings in CWD positive deer. By testing for these bacterial exposures and understanding the process bacteria use in gaining entry through the cell lipid membrane via their pore forming toxins demonstrates the negative impact to the normal lysosome function and proper immune response.

In another early bacterial research study into the possibility of bacteria being a causal agent was concluded that the infectious agent responsible for TSE disease cannot be a spiroplasma or any other eubacterial species. Eubacterium is a genus of Gram-positive bacteria in the family Eubacteriaceae. In this research review most gram negative bacterial screening primers were not used. This would have been important to know since other areas in this review noted that bacterial antigens or toxins were not reviewed during this study but were considered a hypnotized potential if studied. (8, 9)

Another early research review of bacterial use was to inject a spiroplasofma into raccoons. The results of this study indicate that *Spiroplasma mirum* does not induce TSE-like disease in raccoons. (10)

These past research endeavors were the best research approach of their time but with improvement in scientific knowledge and with newer more advanced testing techniques in today's science a relook at bacterial infections leading to CWD in a cervid is warranted.

Today's research advancements have provided multiple improved testing platforms with a greater point of accuracy and detailed analysis. Since the whole genetic genome of the whitetail deer has been completed one area of current interests is reviewing certain genetic markers in farm raised deer for the prospects of selective breeding. This new area of genetic research would provide for the potential development of an improved resistant cervid model through breeding certain deer with a reduced risk for developing CWD. (11)

In the most current genetic review, there was a unique genetic marker identified that correlated to deer testing positive for CWD. These deer were identified with a genetic mutation for the lysosomal storage disease known as Mucopolysaccharidosis (MPS) Type VI. This lysosomal storage and processing disease results in a defective protein (i.e. enzyme deficiency) hindering normal cellular function. This mutation is also referenced in the mouse model demonstrating that MPS can lead to amyloidosis, synucleinopathy, and an apparent prion encephalopathy; with the accumulation of misfolded proteins generally considered to be an indirect result of the progressive failure of lysosomal function in inbred mice (12)

Therefore, through the use of genetic identification of a disease process it raises the possibility that CWD may potentially present diagnostically in the absence of an infectious exposure (i.e. sporadically), and that future research should focus on the pathophysiological timing and potentially complex biochemical mechanisms of the disease processes. (11, 13)

Other research efforts have also thought CWD was sporadically generated or it's just spontaneous event as a causal pathway of how CWD originally generates in deer or elk. But, in a medical sense, the definition of a spontaneous process is one without apparent cause of a said disease processes or remissions**.** (14)

So it would seem that a cause or type of trigger would have to be introduced to a control in an action to get a reaction. In past research, exposures or injections of prion materials are the trigger for disease modeling in a research or wild setting. But it doesn't answer the questions to real life events in an animal model of when a spontaneous case presents itself when no one has an answer to how does CWD happen. What's the trigger?

Historically we sought to advance the scientific knowledge using a physiological approach in what disease organisms were found in farmed and wild cervids alike. Once identified, one would be able to then review a potential mitigation strategy for this disease process if one was to exist. The original hypothesis continues to review the following: can a cervid supported with optimized feed, forage and water along with its genetics stave off an initial or continuing disease process

that could lead to the onset of a disease generating process like the neuro-degenerative disease called Chronic Wasting Disease (CWD)?

In our past and concurrent research, a focus has been to review a pathophysiological timing and what potentially complex biochemical mechanisms of disease via bacterial infection in deer and elk. Our research findings, to date, demonstrate how different environmental bacterial exposures, left unchecked, will negatively impact deer / elk. Once these bacteria(s) integrate themselves into the cellular structures of deer or elk they set up shop with their survival mechanisms which reduces the functionality of the host cell that supports the immune system (3). This loss of functionality and continued dysfunction, over time, reduces the animal's ability to mount an immune response to the onset of a disease process we have come to know as CWD.

Through our research, we have provided the pathways and findings how an acute to chronic inflammation (15) process will eventually lead to a cellular diagnosis of amyloidosis. Amyloidosis is defined as a cellular protein miss-folding complex and process in the body that could be localized in a specific organ or tissue whereas resulting in cascading disease processes' describing transformation in scrapies and CWD. (16)

The current genetic research (11) identified a genetic mutation in CWD positive whitetail deer that leads to identification of a genetic cellular lysosomal storage and processing disease mechanism in CWD positive deer. This finding of a mutation in CWD positive deer affecting the normal lysosomal function provides corroborating evidence to our biological findings of bacterial infection of the cells thus leading to the change to prion diseases.
The lysosome physiologically plays an integral part of the intracellular defense system against microbes that when compromised by bacterial infections would lead to intra cellular dysfunction reducing the ability to fight off the disease process. (17) It is also noted that when bacteria invades and alters the lysosome normal function of killing pathogens they alter the lysosomes ability to do so for which supports internal cell survival of pathogens. This could be a contributing factor to the well known clinical problems of a relapsing infection and persistent carriage despite antibiotic treatment. (18, 19)

Another corroborating example of intra-cellular pathogen survivability is found via review of the host microbiota as performed in our current research. The microbiota is defined as a non negligible body component, but is a crucial mediator in modulating cancer susceptibility and tumor progression in addition to the well-known genetic, epigenetic, and stromal microenvironment elements. Microbial organisms (bacteria) exert their functions notably through indirect pathways (including metabolites and the immune system) on distant or proximal tumor tissues, particularly in colorectal cancers where they are in intimate contact with the gut (bacteria) microbiota. However, in recent years, there are emerging lines of evidence that microbes are also integral components of the tumor tissue itself. (20)

In our current bacterial reviews through the use of the microbiome methodology we have previously identified which bacterial organisms were directly tied to the development of the amyloid B-sheet formation (21) and integration into the mammalians body complexes (22) Some of these same pathogenic organisms with pore forming toxins capabilities were reported in other scientific fields but never been reported in cases of CWD positive detected deer / elk in this field of research. This is due in part that it is only required for testing and reporting of CWD when a

deer / elk dies and is only tested for a prion detection. There is no current recognition or monitoring for any other underlying disease process (23) such as we have outlined in our bacterial reviews.

Summary

In this review, limited historical research showed bacteria were thought not to be the root causal pathway to the onset of CWD in cervids. More recent data demonstrates otherwise.

Again, negative bacteria was found in deer on a CWD quarantined farm, and the wild deer population of the endemic CWD area of SW Wisconsin. These negative bacteria were not present on control farms or wild deer in this study outside of the SW Wisconsin endemic area. These bacteria, as outlined, have a direct capacity to reduce the proper immune function of cervids and, in time, allows for cervids to self develop CWD.

We continue to support efforts of the cervid farm industry to build upon farm management practices. The farmer needs to be continually vigilant in monitoring for, reduction of, and elimination of negative bacterial sources that would have a negative impact to your cervids.

Left unchecked in practice, negative bacteria will lead to immunity challenges of cervids. These challenges would result in cervids developing either an acute or chronic health consequence, which negatively impacts cervid health and the farms bottom line, a healthy herd.

Using these pro-active practices would provide an elevated level of continued Bio-Security. Improving surveillance techniques leads to identifying corrective actions needed to be effective in the reduction of negative bacterial loads in cervids when warranted. One important area often overlooked is the surveillance of feed, forages and water sources offered to cervids on the farm. Part of developing your farms best management practice includes effective health care program including vaccines, parasite control. Health care support includes timely preventative care for all cervids including those prior to birth of fawns or calves on the farm. By reducing the potential risks of exposing your deer and elk to bacteria with infectious capabilities of pore forming toxins minimizes a negative health consequence to your herd.

This information provides the cervid farmer and wildlife specialists alike new tools for improving the health management of their herds.

This herd health improvement process has resulted from our supported research. It continues to provide new pathways toward practices, that when used appropriately, will result in positive mitigation against a disease. On the farm or in the wild cervid populations alike these processes will help protect against the herd's development of CWD.

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To review any research reports - contact your Board members re: continuing to fund our CWD research**.**
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