**Understanding CWD and the Deer Breeders’ Efforts to Solve It**

By Dennis Simpson

CWD has been around for more than 50 years. During that time, hundreds of millions of dollars have been spent on research. The research has mostly focused on prion protein and ways to detect the toxic misfolded form in animals and the environment. This has been driven by fear that infectious misfolded protein would be spread across the landscape and increase the prevalence of CWD. This fear generated large amounts of money for research and burdensome regulation on the private whitetail deer industry.

The deer industry itself has spent large amounts of money on their own research and is now beginning to gain insight into the disease process and potential causatives.

One of the ways the industry is now looking at controlling CWD has been to look for genetics which “resist” the infection. This has been done before in sheep and has successfully eradicated the “prion” disease known as scrapie. Animals that were euthanized once a herd was found to have an infected animal were genotyped to see if there was something different about them that kept them from becoming infected even when they were exposed to the disease. Several different variations in genetic makeup have been discovered which appear to inhibit the onset of CWD. Animals with these genetic variations have been bred and are now being tested by the USDA at the National Veterinary Services Laboratories in Ames, Iowa.

To fully understand this resistance, a full understanding of the prion protein must come first. Prion protein exists in all mammals and is highly concentrated in the central nervous system and the brain. It is produced within the cell and, once trimmed (cleaved) to its final form, travels through the cell membrane and exists attached to what is known as lipid rafts which surround the cell. Many believe the final protein serves as a small antenna or conductor which functions to send and receive neuronal communication. One study showed the prion protein actually generated an ionic pulse when cleaved to its final form, potentially setting up communication pathways for neurons.

The protein itself is a chain of different amino acids. This chain contains 254 amino acids bonded together. Each amino acid has an ionic charge of +2, +1, 0, -1, or -2. Traditional genetic code says the ionic charges range all the way up to +4 and down to -4. These charges being in the exact order is what creates the ordered helical shape of the protein. When an incorrect charge is assembled, the helix fails and the protein becomes a disordered flat ribbon (misfolded). This occurs regularly and the body simply digests the faulty protein and makes another. In the case of CWD “infection,” this protein resists the digestion (protease resistant) and remains within the cell where it is said to cause future proteins to take on the same misfolded shape and proliferate “disease” (templating).

When the protein is produced, it is originally 254 amino acids long. This form of the protein is described as two halves: the N terminus (codons 1-110) and the C terminus (codons 111-254). There is then a final processing which trims the chain (cleaving). The first step is undertaken by a zinc metalloprotease. This protease in particular is known as ADAM 10. Adam 10 bonds to the N terminus at or about the 23rd amino acid (codon 23). It is said to bind above +2 charges of amino acids Lysine, Histidine, and Arginine. The protease cuts (cleaves) the first 23 amino acids and they are recycled within the cell where they are used in making new proteins. The second cleavage occurs on the C terminus at codon 230. The fragment which is cleaved is said to remain attached to the outer cell wall. One key note about this zinc metalloprotease is that it operates at 7-10 PH. Typical brain PH in whitetail deer runs 7-7.2 PH.

Once this cleavage is complete, a serine-based protease is activated and another cleavage occurs at codon 110 which splits the N terminus from the C terminus. The C terminus in its final form penetrates the cell wall and becomes attached to the lipid rafts surrounding the cell wall by what is known as a GPI anchor. It is said the balance of the N terminus is further broken down by the serine metalloprotease. Some say it begins to build a new protein at that point. It should be noted that the section which remains with the C terminus containing codons 111 – 134 is known as the toxic area where the misfolding occurs. If indeed the cleaved N terminus from codon 23-110 forms a new protein, it could be viewed as the seed for new misfolded protein (templating).

The N terminus has a location where there are four places that copper ions bind to it (Octapeptide region). Prusner knew there was another metal binding site at codon 110. It is said to be a binding site for manganese or iron. This is where the potential to disrupt the orderly assembly of amino acids that build the helix and cause the disordered flat (beta sheet) structure to occur. Both metals have multiple potential charges (valences). Normal +2 charge can easily become +3, +4, potentially up to +7 depending on their oxidative state which is 100% dependent on PH. So, if indeed an iron or manganese ion is bound at codon 110 and the cellular PH would fall by .5 PH, the ability of the new protein to build as a helix would be inhibited. Some say that the initial cleavage by ADAM 10 (zinc metalloprotease) destroys the toxic area and any efforts to breed resistant genetic variations should be focused on ensuring this cleavage occurs properly.

When looking at the ionic charges of the entire 254 amino acid chain of whitetail deer, the sum of all of them is -11 (sheep were -5). If this original cleavage cuts away this negative charge and leaves an ionically neutral chain, it might stop the bonding together of misfolded protein and creation of areas with no intra cellular fluid and the formation of plaque.

When considering genetic variations which appear to inhibit onset of “CWD,” there are two that are inserted in positions of importance. The (H) histidine +2 substitution at codon 95 replaces (Q) glutamine -2. This potentially changes the cleavage point of ADAM 10 (zinc metalloprotease) from codon 111 to codon 96 as zinc metalloprotease is known to locate next to histidine and its +2 charge. This would then remove codon 110 and its ability to bond multivalent metals manganese and iron and potentially eliminate the templating of misfolded protein as the N terminus build new protein.

The next substitution which has shown promise is the insertion of (K) lysine +2 at codon 226. It also replaces (Q) glutamine -2 and would move the second ADAM 10 (zinc metalloprotease) cleavage down to codon 227 from codon 230 and change the total chain charge to be more neutral.

There is another amino acid substitution which has shown reasonable ability to resist “infection.” It is a serine -1 substitution at codon 96 replacing (G) glycine -2. This substitution not only reduces ionic electronegativity, it also reduces acidity as glycine is the precursor to glycemic acid. Serine is acidic, but less than glycine. When considering resistance, we must look at substitutions as higher expression of that amino acid and consider the possibility these substitutions are elevated throughout the entire genome. When considering the amino acid serine, there are two critical regulatory areas of the body which serine is important. Both the mucosal barrier in the lower intestine and the blood brain barrier are improved with higher serine expression. The particle size which is allowed to pass through either is smaller when serine is increased. This will be important later when potential initiators or causatives are discussed.

So, we have this prion protein which can and does misfold. When conditions are normal, the body easily digests them and the animal remains “undetected” or healthy. However, in some cases, the misfolded protein is insoluble and stays in the cell. If we listen to the “experts,” it can last for decades and cannot be destroyed; it can cause new infection in deer. If we consider the “opinion” of the fear mongers, it can jump the species barrier into humans and it can be flown by scavenger birds for thousands of miles, infecting animals for generations to come. Very scary indeed.

We have begun to study herds of deer that have had “positive” animals and are beginning to accumulate common denominators between them. There are now reasons to believe the causative agent is not the prion the deer shared at the water source or feeder or the bird poop that contained and “infectious prion” that dropped on the soil and was absorbed by a plant which was later consumed by the deer.

Blood work has been done on herds where positive animals are found and it shows very clear evidence that the herd was having issues as a whole and the deer that were positive had the same issues but were at higher indication levels. The herds all suffer from metabolic acidosis (low blood PH).

We have done complete blood work on groups of deer from positive farms. The liver, kidneys, and spleens were checked for mineral content on some groups. Brain PH was checked immediately postmortem and found to be 6.2-6.4. Normal brain PH is 7-7.2. One interesting note is fallow deer who cannot get CWD have a blood PH of 8!

There was a paper written in 2018 that said if PH falls .5 PH, the prion protein misfolds and aggregates. So, we can confirm that in whitetail deer that are positive, we have found brain PH of 6.1. Interestingly, a positive herd in Texas has a low sodium-to-potassium ratio which not only causes acidity it reduces H+ entry into the cell. H+ is critical to serine metalloprotease. That herd was also very low in zinc.

Other biomarkers which were elevated were Spleen iron, CK, AST, and anion gap.

Spleen iron levels were triple normal high limits. The function of the spleen is to breakdown dead red blood cells and recycle the metals. The high iron indicates high volumes of dead red blood cells as the liver, kidney, and blood iron levels were fine. The problem with the pattern is the blood cells along with muscles and organs are seeing the oxidative stress of dysfunctional kidneys and a contagion which is causing metabolic acidosis. The spleen is very vulnerable to certain infections like ecoli, mycoplasma, and hepatitis.

CK is an indicator for multiple infections, but it becomes elevated when red muscle or red blood cells are under attack. CK numbers being high is a bad situation. The damaging or die-off of red tissue cells and blood cells is a problem for the spleen, kidneys, and liver. The filtering mechanisms by which they function can become overwhelmed by the volume. They dysfunction in the start of the process, but over time would fail completely.

AST is a liver enzyme that is released when the liver is damaged. The liver is still regulating body mineral and metal~~s~~ levels but is showing signs of the oxidative stress of the low PH and is heading toward potential organ failure.

Anion gap is basically a PH indicator. If the level is high, the animal has metabolic acidosis or acidic blood.

The next step is to tie in the metabolic conditions to infections in an effort to find the causative. I have three suspects and one fits the bill 100 percent. They are ecoli, hepatitis, and mycoplasma. All three have the potential and we may find all three are causing CWD. Maybe this will be the different strains of CWD we have been told about?

**Ecoli**

Ecoli heads the list of causatives for a lot of reasons.

Ecoli is a very powerful chelator of metals. When this occurs, ionic charges change and that perfect orientation of charges that supports the ordered helical shape seen in noninfectious prion protein is lost.

Ecoli infections can produce Hemolytic Uremic Syndrome (HUS). Ecoli produces Shiga toxins which cause HUS. HUS causes kidney failure. It causes breakup of red blood and tissue cells. This breakup overwhelms the kidneys and clogs off the filtering mechanisms of both the kidneys and spleen. When we have this condition, kidney function slowly decreases and regulation of electrolytes is lost causing acidic blood. We see extremely high CK numbers in the herds where positives are found and insane high numbers in positive animals.

Ecoli scavenges iron into cells where it is present. This happens as ecoli infection causes Enterobactin production which is the iron scavenger. When ecoli scavenges iron at the normal +2 charge, it oxidizes that iron and converts it to +3. This form of iron is highly toxic as it causes oxidative stress. Iron at +3 can easily combine with chloride to become iron chloride which is toxic to the liver, kidneys, and blood. Two farms that were positive showed elevated chloride in the blood. Extremely high iron in feed and or water is present in most positive farms. Not to say normal levels of iron are not susceptible to being used by ecoli to create Fe+3 and accumulate it in the cell. As the iron builds within the cell at a more positive ionic charge it can now compete with copper for the binding sites in the octapeptide region of the N terminus. It also has a greater ability to bind to the 5th binding site at codon 110 where we know iron is preferred. If indeed the N terminus section running from codon 24-110 is used in production of new prion protein, we now have the condition where the new prion protein will be produced with improper charge orientation to produce helical shape and will be made as disoriented flat sheet (templating of new protein to be “infectious”). Studies have shown that animals infected with CWD had stability of the misfolded protein when iron was present and that those proteins were resistant to digestion by protease (zinc metalloprotease). If the ionic charge of the iron in the cell is already +3 valence, the templating is bound to happen.

Go back to the positive indicator used in CWD testing having a -3 charge and “infectious” prion protein having +3 iron bonded to it. It would seem natural that any cell with ecoli infection and high concentrations of iron +3 might cause the positive indicator and its attached dye to light up.

One more interesting thing is the lymph nodes are where we test for “CWD.” The lymphatic system function is to filter bacteria and viruses from the body.

Ecoli is a known zinc and serine metalloprotease inhibitor. Go back to our crucial enzyme zinc metalloprotease (ADAM 10) and its cleavage being critical to producing proper shape of mature protein. You could see how ecoli could be implicated. Think about reducing the capability to digest the cleaved fragments of the N and C terminus with serine protease. This reduction would be seen in fragments building in the lymphatic system. This inhibition of enzymatic activity is caused by acidic conditions. Remember, we are losing the function of the kidney and thus the ability to regulate PH.

Ecoli can damage the pancreas. The pancreas regulates glucose in the body. We saw a 30 percent reduction in glucose count in a positive herd as compared to an undetected farm only a few miles away. Both farms were on the same feed but had different water supplies.

Ecoli can cause pneumonia. One positive farm said their deer have had a nagging cough.

Of five positive farms where water testing was done, there were high ecoli, coliform and iron in the water. Some farms had particular pens where CWD was generated when CWD was not present anywhere else on the farm. They had ponds or seasonal runoff water accumulation areas. All of them were high in ecoli, coliform, and iron. High iron promotes bacterial proliferation of ecoli, mycoplasma, and tuberculosis. When these things proliferate in cells high cytokine production occurs. This is an inflammatory condition and would produce high CK readings in blood work.

Many research papers have noted that tetracycline and doxycycline were effective ~~any~~ in slowing the progression of CWD. There are very few forms of ecoli that have been found to be resistant to tetracycline.

One positive herd had low zinc. Zinc inhibits transmission of ecoli infection to organs and the central nervous system.

Ecoli in humans has its own encephalopathy. CWD is known as transmissible spongeform encephalopathy!

Ecoli has many forms and the exact form needs to be identified from samples taken at positive farms. If indeed they are all the same type, the loop could be closed and we can begin to take steps to mitigate the ecoli infection. I believe resistant genetics are more about cleavage points and resistance to ecoli. We could also investigate vaccination possibilities that might stop it.

The counties where CWD prevalence remains consistently higher in Michigan are Kent and Montcalm counties. The Flat River Drainage has the particular townships of both Kent and Montcalm where CWD prevalence is high within it and also includes part of Ionia County. That drainage is documented as one of the highest ecoli polluted in the state by the DEQ. Ecoli levels in the Flat River are equal to or in excess of what we have seen in the positive farms.

**Mycoplasma is another potential for a strain of “CWD”**

Mycoplasma shares the ability of ecoli to produce hemolytic uremic syndrome. So, all of the issues with acidity caused by kidney failure, loss of liver and spleen function, and misfolding of prion protein are there. One positive herd was noted as all the animals coughed.

**Hepatitis is another potential candidate in a viral form. (A and E)**

Hepatitis would damage the liver first. In the blood work from positive farms we see high AST. Remember, AST is a liver enzyme which is leaked from the liver when it is damaged. Waterfowl carry viral hepatitis. Draw a parallel between positive farms (pens) having ponds, seasonal flooding, and waterfowl. Think about CWD showing up in Norway or Finland far away from traditional CWD areas. Seasonal migration of waterfowl could bring in this contagion. Moose now share the ability to catch CWD. Moose forage in the waters where waterfowl feces are common. Hepatitis causes encephalopathy.

Studies of the Flat River Drainage in Michigan’s CWD zone showed high levels of goose and duck feces identified by DNA type in their ecoli studies! It is interesting that CWD showed up in Michigan in 2015. In 2016, Michigan had a massive outbreak of Hepatitis A in humans. Kent County now has the highest vaccination rate in humans against Hepatitis A.

**So, what are our options??**

First, we continue to breed resistant genetics and let the test at Ames, Iowa play out. The Ames test is a USDA run project where we have KK, KH, KS, HS, and SS genotype fawns housed with CWD positive GG genotype fawns. They have been in the facility since mid-February. They had the positive animals introduced to the pens in March. If the resistant genotype animals can fight off “infection” then we have a breeding strategy to work with. Dr. Seabury’s method of genotyping is not in play there. So, we must get another trial going using animals identified resistant by his method. All the animals currently at Ames are genotyped based on Dr. Haley’s approach. It would be wonderful if we could get Dr. Seabury samples of the fawns at Ames to have his rating on the susceptibility of those animals. We know that markered animals have higher blood ALP readings indicating a more basic PH.

Second, we get further into the type of ecoli we are encountering, check animals at positive farms for titers to Ecoli, hepatitis, and mycoplasma to confirm their presence.

Third, we capitalize on humic acid feed supplementation and pen “disinfection.”

We did a study this year where we took soil samples from a positive pen (one of those with a pond). Samples were tested by Davin Henderson and he found several samples he could confirm were “infective.” Coincidentally, they were from right beside the pond! The samples were treated with a small amount of humic acid and were no longer detectable as “infectious” after 72 hours. A positive lymph node was then tested. It was confirmed “infectious” and treated with a small amount of humic acid. The test following the humic acid treatment showed again the “infectivity” was gone.

We have done a trial where we rectal biopsied over 100 animals from a positive farm and found six positives. They were split in six pens and the undetected animals were split up between the six pens. Normal feed was fed to three pens and feed supplemented with 12 pounds per ton of humic acid was fed to the other three pens. We were only allowed about 70 days of feeding before depopulation occurred. At the point of depopulation, the positive animals on normal feed were clinical and one had died. The positive animals on humic acid supplemented feed were fat and healthy. Those animals still all tested positive, but they were not deteriorating in body condition and this test went through the rut!!

We now have another farm where positives are identified with rectal biopsy and are being fed humic acid. There is no time limit here and the animals are in fine condition. The bucks are successfully breeding, the does produced healthy fawns, and mortality is extremely low. The pens have been treated with humic acid and the feed is supplemented at 30 pounds per ton. We will again be doing blood work, rectal biopsies, drawing semen, and AI’ing the does this fall. Fecal samples of over 100 deer from this farm have been sent out for “infectivity testing.” If the fecal samples of the positive deer show no “infectivity” when being fed humic acid, shedding is eliminated.

Humic acid is a known to sequester iron. It makes the iron unavailable for the ecoli to scavenge. Iron at +3 charge is absorbed by the humic acid and the toxicity is alleviated. This stops redox activity and the accompanying oxidative stress.

Humic acid as a soil supplement holds tremendous potential to make foraging much less of a risk to picking up “infectivity.” When we think of hunters’ food plots being a safe zone for deer to forage, we now have a huge reservoir of players to contribute to solving CWD. If in the end we can prove that humic acid is viable in the wild herd, we might be able to manufacture a supplemental feed in pellet or block form where humic acid can be included and even a vaccine for the contagions. This would then be a reason hunters would be allowed to bait!!

At this point, we need to look at funding further research. I have personally sponsored a lot of the money and the deer breeders’ organizations have contributed the rest. We need to work on government-sponsored research of these common denominators seen in the private herds for the benefit of the wild deer. I wonder if it might be a viable option to advise the hunting public of our discoveries and start a go-fund me page for them to assist us in the final work???

There are quite a few people who have helped me along the way and I am so grateful to them all. Dan and Cindy Harrington especially. They have been the lead investigators in humic acid research and allowed me countless hours of time to bounce ideas off them. They are both currently working on a follow up to the short bit of information I have included on humic acid. Tim Condict has travelled many miles with me and on his own setting up projects and collecting samples. He has asked questions of me that inspired me to dig deeper on many occasions. Shawn Schafer and the team that does the Washington fly-in got us the funding for Ames, Iowa. Keith Warren has helped me get the word out and defended me from critics when I needed it. Kevin Grace has given us darts and got me fallow deer blood samples. Alex Draper spent many hours working with me in the field and writing up reports. Jacob Glick, Ron Douglas, Chris Hayes, Gina Bozzer, and Jim Moses all contributed semen to breed fawns for Ames. Gary Edwards helped with fawns for Ames as well as Chris Hayes, Gina Bozzer and Dan Harrington. Laurie Seale has been so important in setting up projects. Scott Follett has been so important in getting us access to animals and samples. Greg Flees has spent a huge amount of time talking to me about conditions he has seen with positive deer and given me the opportunity to get samples for multiple tests. The DBC, UDFoM, and WOW have all spent money on research and, without this team, we would be nowhere near where we are today. All of the people at Texas Parks and Wildlife, USDA, and local veterinarians are so critical in this effort and we owe them all a debt of gratitude. There are so many that have contributed, it is hard to begin to list them all.

We are almost there and it has been a wild ride so far. I look forward to seeing all the breeders at events and just talking about our deer and our families with no mention of CWD someday.

My best to all who love deer,

Dennis