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Review of Current Science

Concerning Genetics and Chronic Wasting Disease (CWD) for North American Elk, Whitetail Deer and Mule Deer

July 2018

Introduction & Background

Introduction & Background

This review of the published scientific literature was produced at the request of the Texas Deer Association (TDA) and the North American Elk Breeders Association's Elk Research Foundation (also known as the Elk Research Council) for the purpose of explaining in plain English and with a minimum of scientific jargon about what is known, what is not known, and implications concerning the influence of genetics and Chronic Wasting Disease (CWD).

As such, this review is not in the normal format of a scientific journal article nor is it meant to be. This document is, however, based solely on published science as of July 2018. There have been many scores of articles published in peer-reviewed journals on various aspects of genetics and CWD, but these generally are not critically read (or understood) by producers of deer/elk, wildlife biologists, the public, makers of policy, and definitely not by the press or media.

This document is also not intended to be a comprehensive review of all the aspects in the scientific literature concerning genetics and CWD, but rather emphasizing what is known currently about susceptibility/resistance, gene frequency, and genetic selection in some species of North American Cervidae, the short-comings of the known science, as well as future research needs also will be discussed.

Introduction & Background

Specifically, TDA and the ERF requested that certain questions be answered.

These are listed below:

1

Relationships with susceptibility to Chronic Wasting Disease in North American Elk, or White-tailed deer with specific alleles of the normal cellular prion protein, encoded by the PRNP gene.

2

Known incubation periods in natural and experimental situations for all known genotypes of North American Elk and White-tailed Deer.

3

Understanding when the different genotypes and their exposure to CWD versus their time to become infected. Are certain genotypes less susceptible or is their disease progression slower?

4

Understanding the differences between animals that become clinically CWD positive after shorter exposure times compared to animals with a prolonged incubation period.

5

Estimated or known frequency of different genotypes of North American Elk, Whitetail deer in domesticated and free-ranging population.

6

Known prion shedding that may take place by animals exposed to CWD with prolonged incubation periods. Understanding shedding in different stages of incubation periods.

7

How different genotype animals have the opportunity to impact whether a herd becomes CWD positive? Particularly for known CWD positive states and provinces.

8

What scientific shortfalls exist in understanding more about CWD resistance and where should future research be focused?

NOTE: With permission of TDA and ERF the editors added mule deer to the above questions to be answered in the document.

GENETICS 101 REFRESHER

Genetics 101 Refresher

Even with every possible effort made to avoid the use of scientific jargon and overly technical information, genetics is by nature a technical science. Therefore, to assist and enable the reader to achieve a more clear and easier understanding of the following document, a brief overview of genetic definitions and concepts may be necessary in general and more specially useful in the section on the discussion of scrapie in sheep and goats.

The basic building blocks of the genetic code are nucleotides. Nucleotides are made up of 3 subunits. The nucleotides make up the structural units for DNA and RNA. A codon is a set of 3 nucleotides and the codons specify the type and sequence of amino acids. Amino acids combine in the sequence to form proteins. Therefore, proteins are formed by hundreds of codons specifying hundreds of amino acid sequences. There are 256 codons (and 256 amino acids) on the deer prion protein.

Genes are the basic physical and functional unit of heredity. Genes are made up of distinct sequences of nucleotides (DNA) which act as instructions to make the various proteins as described above. Genes may vary in size from a few hundred DNA bases to millions. The human genome is estimated to be around 25 million genes.

Every individual animal has 2 copies of each gene. One copy is inherited from each parent. If the 2 gene copies are the same then that gene is termed homozygous. If they differ the genes are called heterozygous. Each of the two copies of the gene also may have two or more alternate forms that arise by mutation These alternate forms are called alleles. The various forms of the alleles are usually represented by two letters such as HH, or MM when discussing genotypes. The HH and MM pairs would be called homozygous and HM would be called heterozygous. The letters represent various amino acids such as S for serine or G for guanine. The locations of codons on the protein are identified by a number. So, for example, in elk codon 132LM means at location 132 on the prion protein it is heterozygous with amino acids leucine (L) and methionine (M).

Even with this very brief summary of genetics, it is easy to clearly see that it is rather complicated. Also, it is easy to see why it is generally very difficult to identify specific genes that control a particular biological process such as resistance to a disease.

SELECTION FOR PRION GENETIC RESISTANCE – SCRAPIE IN SHEEP AND GOATS

Selection for Prion Genetic Resistance – Scrapie in sheep and goats

In terms of CWD, the primary objective of possible selective genetic breeding in elk, WTD, and MD is to identify genotypes resistant to the disease. This strategy is basically to copy the process in sheep where genotypes were identified that resisted infections to another similar prion disease called scrapie. To be able to emulate the process utilized in sheep and scrapie resistance, deer breeders should have a very clear and through understanding of what is involved and the complexities of reaching that goal.

This next section is rather technical and complicated but it thoroughly outlines in detail what must be understood and achieved by the farmed deer/elk industry if they wish to go into a successful selective breeding program for CWD resistance that would emulate that which was accomplished in sheep and goats.

The following discussion is from "A New Tool: Genetic Scrapie Resistance in Goats" by Stephen N. White PhD, David A. Schneider DVM, PhD, DACVIM (LAIM)

Scrapie eradication requires a joint effort in sheep and goats. Genetic scrapie resistance has been an important tool available to the sheep industry for decades but not to goat industries until recently. Two goat prion gene alleles have now been shown to confer resistance to classical scrapie . They are S146 (serine [S] amino acid at prion protein position 146) , and K222 (lysine [K]at position 222) . Goats bearing just a single copy of either one of these alleles have been strongly resistant to infection during natural outbreaks as well as direct challenge experiments.

Over the last 15 years (2002 - 2017), the European Union (EU) has recorded more than 10,5 00 cases of scrapie in goats. To address this problem for eradication, the EU formally requested that the European Food Safety Authority (EFSA) evaluate the strength of evidence for genetic scrapie resistance in goats. The EFSA brought together a panel of European experts to conduct a comprehensive review of research. In its recently published review, the panel concluded that today's evidence for genetic resistance conferred by the S146 and K222 alleles in goats exceeds the evidence that was available for R171 when it was recommended for resistance in sheep. Thus, the commissioned review recommended the use of genetic scrapie resistance in goats to augment eradication programs.

Rules for implementing goat genetics in scrapie eradication programs were left to each European country to develop, but final rules are not yet available. While scrapie resistance alleles in goats have not been formally recognized in the U.S., the National Scrapie Eradication Program plans to conduct a herd cleanup pilot project in goats based on S146 and K222 goat alleles similar to that done for sheep in the early days of genetic resistance in sheep .

Selection for Prion Genetic Resistance – Scrapie in sheep and goats

Goat DNA testing services for S146 and K222 alleles are available at the Veterinary Genetics Laboratory (VGL) of UC - Davis. Details may be found at this website: https://www.vgl.ucdavis.edu/services/GoatScrapie.php

Reduced pricing has been arranged for testing done through VGL for members of two of the largest goat organizations (American Dairy Goat Association and American Boer Goat Association).

Similarly, testing services are in development at NeoGen Genomics, Inc. (GeneSeek). Service details will be available at this website:

http://genomics.neogen.com/en/research - and - development - genomic - discovery#sheep - and - goat

USDA has not established an approval process for laboratories to conduct official scrapie susceptibility genotyping in goats, so testing at either lab would not be considered official testing for regulatory purposes.

Both S146 and K222 are naturally occurring alleles in U.S. goats. The S146 allele is common in U.S. goats and has be en identified in 7 out of 10 breeds of both meat and dairy types, including: Boer, Tennessee fainting goats (myotonic), Nubian, Alpine, Saanen, LaMancha, and Pygmy goats. Among these breeds, the S146 allele is particularly common in Boer and Nubian goats. S146 is probably present in additional breeds and will likely be found as larger numbers of goats from those breeds are tested.

The K222 allele is most often observed in dairy breeds. One U.S. study identified it in Toggenburg and LaMancha goats. Other studies have identified K222 in most European descended breeds, including Alpine, Saanen, and Anglo - Nubian. The K222 allele is probably present in additional breeds, too, and will be found as larger numbers of goats from those breeds get tested.

As with sheep, goat producers are encouraged to maintain overall herd health, productivity, and reduce inbreeding by selecting goats superior for many traits (not only scrapie resistance) and from diverse families. By using such measures, goat producers can boost or add scrapie resistance while continuing to improve all other aspects of goat breeding quality . Overall, breeding for strong scrapie resistance in goats as well as in sheep will provide one more tool to combat scrapie.

Selection for Prion Genetic Resistance – Scrapie in sheep and goats

Now in regard to any potential CWD resistance programs in cervids, it should be noted that:

1

The genes for resistance to CWD infection have not been identified as they were for scrapie in sheep and goats.

2

There is no Federal CWD Eradication Program; there is not sufficient data on CWD resistance/susceptibility based on experimental infections and natural outbreaks.

3

There are more susceptible cervid species to CWD as compared with scrapie in just sheep and goats; there are no official labs for CWD/DNA testing of deer and elk.

4

CWD is found in species of deer and elk both in farmed and free-ranging populations and this situation does not occur in scrapie.

GENETIC STUDIES ELK (*Cervus elaphus nelsoni*)

Genetic Studies Elk (*Cervus elaphus nelsoni*)

One of the studies on the frequency of prion protein (PrP) genotypes of free-ranging elk in three populations in Colorado was done on 171 samples collected between 2002 and 2005 and was conducted by Perucchini et. al. (2008 J Gen Virol). The PrP gene is polymorphic (two or more variations can occur) at codon 132 either methionine (M) or leucine (L). In the 124 apparently CWD un-infected elk the frequencies were 65.3% MM132, 32.3% ML132, and 2.4% LL132. For the 47 CWD infected elk, the frequencies were 70.2% MM132, 27.7% ML132, and 2.21% LL132. These data in this study indicated the genotypes in both CWD infected and apparently normal elk were not different, and all genotypes showed the same susceptibility to infection.

In an excellent review of "the role of genetics in chronic wasting disease of North American cervids" Robinson et. al, 2015 (Prion) stated in the section on elk (page 9), that there is strong evidence for delayed progression in elk with the 132L allele. The incubation time from oral exposure to clinical signs is twice as long in LM132 as that seen in MM132 elk (Hamir et. al, 2006, J Vet Diagn Invest). O'Rourke et. al in 2007 (Neuroport) reported that LL132 further extended incubation times. Research was not conclusive if L132 also resists infection.

In 2017 Haley and Richt, on page 6 of their paper in Pathogens on "Evolution of Diagnostic Tests for CWD, A Naturally Occurring Prion Disease of Cervids", stated that data from free-ranging and experimental studies in deer and elk showed that "… the hosts prion gene (PRNP) sequence may modulate susceptibility." However, later studies showed that all of the 132 alleles are susceptible to infection, and they are only associated with progression of the disease and incubation periods from infection to clinical disease.

Moore et. al (2018 BMC Vet Res) experimentally exposed eight elk calves at eight months of age by oral inoculation. The calves were from different game farms. The inoculum was pooled from two infected and clinical elk (one MM132 and ML132) from a game farm. There were four LL132 calves, two ML132 and two MM132 orally exposed (inoculated) with 3 ml of inoculum for five consecutive days (a total dose equivalent to 15g of pooled brain).

The incubation period from time of inoculation until clinical signs of CWD varied between genotypes. The two MM132 elk were clinical at 23 months post inoculation (mpi), the two ML132 at 38 and 40 mpi, and the four LL132 at 59mpi, 63 mpi, and two at 64 mpi. All eight elk, however, became infected and died of clinical CWD.

There were differences in spongiform changes between genotypes. Encephalopathy was more prominent in the gray matter in MM132 and ML 132 while in LL132 elk white matter was more affected.

Genetic Studies Elk (*Cervus elaphus nelsoni*)

In all genotypes, prions were abundant in lymphoid tissues of tonsil, retropharyngeal lymph nodes, and lymphoid tissue in the gut. Skeletal muscle, diaphragm, bladder, lung, liver, and several other tissues were negative at death in all samples examined. There was less accumulation of CWD prions in those elk with longer incubation periods. There were also biochemical differences between MM132 and ML132 when compared to LL132.

GENETIC STUDIES IN WHITE-TAILED DEER (*Odocoileus virginianus*)

Genetic Studies in White-Tailed Deer (*Odocoileus virginianus*)

Kelly et. al 2008 in Prion examined PRNP genotypes for 76 white-tailed deer (WTD) from Northern Illinois that previously had been tested positive for CWD and 120 CWD test negative (actually tested as CWD not detected). The two sets of free-ranging deer were selected to control for age, and geographic location. While all observed polymorphisms (except 2 rare ones) were observed in both positive and negative animals, there was a significant distribution of alleles at 5 loci between infected and noninfected (CWD not detected) individuals. Four of the 5 nucleotide base changes 60C/T, 285A/C, 286G/S, and 555C/T were found at higher frequencies in CWD test negative (test non-detected) individuals which was suggested by the authors as disease resistance. The remaining nucleotide base 153C/T was observed more frequently in CWD test positive animals. These data were interpreted by the authors to be suggestive of CWD disease "susceptibility".

The genetic susceptibility to CWD was studied in 2009 by Blanchong et. al (Infect Genet Evol) in WTD from a CWD endemic area of south-central Wisconsin. The 159 female deer were case–controlled and identified to be closely related. Test positives (n=68) and 91 CWD test negative (really not detected) also were matched for location, and age. Four amino acid genotypes of the 95QH and the 96GS loci (QQ/GG, QQ/GS, QQ/SS and QH/GG) were studied. The results indicated only genotypes with at least one glycine (G) had an elevated risk of infection.

In their 2012 review article on CWD in North American cervids (Robinson et. al, Prion), the authors cited several landmark articles on CWD in WTD. Johnson et. al 2006 (J Gen Virol) documented that brains collected from harvested free-ranging deer, the 96GS deer had less PrPRES accumulation in their brains than the 96GG deer. Under controlled experimental conditions and high dose oral inoculum, the 96S showed a delayed progression of the disease and an increased incubation period. In the same experiment 95H deer had an incubation period twice as long as 95Q deer (Johnson et. al, 2011, PLOS). In several populations of free-ranging WTD, a lower disease prevalence was observed in all populations with 96S which were lower when compared to those with 96G (Robinson, et.al, 2011, Ecol Applied). Lower infection rates have also been observed in the more rare alleles 95H and 115G. In a farmed herd in Nebraska, 116A deer were twice as likely to be infected as 116G deer (O'Rourke et. al, 2004, J Gen Virol), but both genotypes were infected.

A larger and more comprehensive study was published by Brandt et. al in Prion in 2015. CWD susceptibility (test positive) was investigated in WTD harvested by hunters and killed during governmental culling from 2002-2010. PRNP sequences were determined by PCR on 703 deer. There were 579 deer tested for CWD of which 105 tested positive and 474 deer were CWD test negative (not detected). The results of this study did not find reduced susceptibility to CWD in deer with 95H while

Genetic Studies in White-Tailed Deer (*Odocoileus virginianus*)

in previous studies 95H was only found in CWD test negative deer and interpreted as disease resistance. The presence of 96S has been reported to be associated with a longer disease progression and a longer life span in farmed WTD but 96S does not seem to affect the rate of prion shedding from infected animals (Kuznetsova et. al, 2014 Prion).

Haley et. al, 2017 (J Gen Virol) stated that resistance to CWD is manifested "… as decreased prevalence and/or delayed progression of disease". The mechanisms of resistance are poorly understood" but "thought to depend primarily on the host's cellular prion protein (PrPC) amino acid sequence encoded by the PRNP gene" (Lloyd et. al, 2013, Current Opin Genet Dev). The preponderance of the genetic investigations has been centered on PrPC position 96 specifically on glycine (G) or serine (S) that has frequently been associated with susceptibility to CWD infections. The 96S allele is found in about 20% of WTD in the US. The less well studied codons 95 histidine (H) and 116G have been suggested as being involved in susceptibility/resistance.

GENETIC STUDIES IN MULE DEER (*Odocoileus hemionus*)

Genetic Studies in Mule Deer (*Odocoileus hemionus*)

The prion protein (PrP) gene was determined for 1,482 free-ranging mule deer (MD) from Wyoming and Colorado (Jewell et. al, 2005, J Gen Virol). A total of 112 deer of 1,119 (10%) were found to have genotype 225FF or 225FS and only one of those (0.9%) was found to be test positive. Of the1,370 with genotype 225SS, there were 289 positive (21.1%) for CWD. The total frequency of 225SF and 225SS genotypes was 9.3 % while among CWD test positive deer the frequency was 0.3%. The odds of infection for as 225SF deer were 30 times less than the odds of infection for a 225SS deer. In the same report, previously experimentally infected deer were analyzed, and 10 deer with 225 SF genotypes were found to be infected. The authors concluded that the 225SF genotype was associated with longer incubation periods than genotype 225SS.

The accumulation, distribution, and timing of abnormal prion protein were studied in 19 mule deer after experimental oral inoculation. From 90-785 days post inoculation, PrPCWD was rapidly and widely distributed in lymphoid tissues. PrPCWD was later deposited in the central nervous system (CNS) tissues and found "… sporadically in a variety of tissues and organs in terminal disease stages." Spongiform encephalopathy was seen later than the disposition of PrPCWD in the CNS. Deposition of PrPCWD was similar between PrP genotypes 225SS and 225SF. The timing of accumulation of CWD in lymphatic tissues was slower in 225SF deer than 225SS deer (Fox et. al, 2006, J Gen Virol).

Susceptibility to infection by environmental exposure was examined in six mule deer with two different genotypes. Three deer were 225SS and three were 225FF. All three 225SS were IHC tonsil biopsy positive by 710 days post-exposure (dpe), and were showing CWD clinical signs by 723-1,200 dpe, as well as "… gross and microscopic pathology, enzyme-linked immunosorbent assay (ELISA) results, and IHC staining typical of prion disease in mule deer". On the other hand, while all three 225FF deer became infected and died at 720, 924, and 1,783 dpe, they consistently were negative on biopsy, and their clinical signs were "…more subtle". More interestingly the 225FF deer were "suspect" by ELISA at death, "…but showed negative or equivocal IHC staining of lymphoid tissues; both clinically affected 225FF deer had spongiform encephalopathy in the absence of IHC staining in the brain tissue" (Wolfe et. al, 2014, J Wildl Dis).

The importance of the shorter time of distribution and accumulation of PrPCWD into the lymphatic tissue and longer incubation periods differences between genotypes is pointed out by both Fox et. al (2006, J Gen Virol) and Haley et. al (2011, Virolgy). Both have the potential effect of increasing and prolonging shedding before becoming clinical.

COMMENTS ON THE LIMITATIONS OF THE NOTED GENETIC RESEARCH

Comments on The Limitations of The Noted Genetic Research

Experimental research on CWD in cervids generally is very difficult both in terms of the lack of suitable and adequate bio-containment facilities, the length of the disease process (several months to years), the complete lack of a standard challenge (exposure protocol), sufficient numbers of CWD naïve animals, and adequate funding to support quality research.

A quick look at the research cited and discussed in the above literature review emphasizes these difficulties. For example, the majority of what has been done experimentally and therefore what we know about CWD in elk with any certainty was done on only eight elk calves (Moore et. al, 2018). Overall it is an excellent study and ground breaking in terms of pathology and biochemistry, but it had its limitations even as the authors noted. One of the most serious limitations is the small numbers of elk and their sources. The four 132MM and 132LM calves were from a game farm that had 79 cases of CWD. The four 132 LL were from a different birth cohort and from a different location. The other serious issue is the oral inoculum. The eight elk calves were orally inoculated with 3 ml of pooled brain material daily for five consecutive days for an equivalent dose of 15 grams of CWD infected brain tissue. That is a huge dose that is probably never occurs in elk calves under natural circumstances. Also the inoculum did not contain any brain tissue from any 132LL CWD infected elk and that may have affected the final results.

Experimental research on other infectious diseases such as brucellosis (Bevins, JS, Blake, JE, Adams, LG, Templeton, JW, Morton, JK, Davis, DS.1996. The pathogenicity of *Brucella suis* biovar 4 for bison. J Wildl Dis.32(4):581-585) or bovine tuberculosis (Mackintosh, C. G., K. Waldrup, R. Labes, G. Buchan, and F. Griffin. 1995. Intra-tonsil inoculation: an experimental model for tuberculosis in deer, p. 121–122. In F. Griffin and G. de Lisle (ed.), Tuberculosis in wildlife and domestic animals. Otago Conference Series no. 3. University of Otago Press (Dunedin, New Zealand) have documented numbers of bacteria or viruses for use as an inoculum. For example, for over 50 years, the standard challenge (exposure) dose for *Brucella abortus* experiments is $1x10^7$ colony forming units of B. abortus strain 2308 which are dissolved in 100 microliters of PBS (saline) and 50 microliters is placed onto the conjunctiva mucose membrane of each eye. This standard precise dose and experimental exposure protocol allows for comparison of one experiment to another and one animal to another.

Presently there is no standard experimental exposure dose for CWD prions, and there no standard experimental protocol for the route of inoculation in CWD experiments. There are several recognized consequences of different inoculum or route and method of exposure. Currently there is no known or standardized minimum dose of prions or homogenized brain that has been documented for CWD laboratory inoculation. This information

Comments on The Limitations of The Noted Genetic Research

is necessary to more fully understand the incubation period and progression of CWD. An overwhelming inoculation may not present a true picture of the disease progression or may skew the timeline of the disease progression so that it may not represent the rate of transmission in nature.

There is currently no information concerning the possible use of different strains of CWD in experiments. Differing strains of scrapie exist (Bruce, ME. 2003. TSE strain variation: an investigation into prion disease diversity. Brit Med Bull, 66(1):99-108) and are known to cause different pathologies in sheep and mice. If CWD has a spontaneous form, it would be important to understand how it arises and if it is transmissible to other animals.

Many of the genetic studies in regard to "resistance and susceptibility" to CWD were done on free-ranging deer and elk. There are several inherent problems with doing that type of research in free-ranging animals. Those include total unknowns such as: time of exposure; the number of exposures; the age of the animal at exposure; the number of prions in the exposure; the route of exposure; the strain or strains of the prions in the exposure; and the overall physiologic status of the animal. These unknowns can and probably do have serious influences on the resulting data and its analysis. For example, not knowing when a free-ranging deer or elk was exposed to an infectious dose of CWD, then the incubation period cannot be calculated.

It is for the reasons listed above, some of results of earlier published studies on genetic resistance to CWD in cervids that were based either on small numbers of experimental animals or done on free-ranging animals, have led to erroneous conclusions and ineffective policy decisions.

SUMMARY - GENETIC RESISTANCE AND/OR SUSCEPTIBILITY TO CWD IN ELK, WHITE-TAILED DEER (WTD), AND MULE DEER (MD)

Summary - Genetic Resistance and/or Susceptibility to CWD in Elk, White-Tailed Deer (WTD), and Mule Deer (MD)

None of the genotypes examined in elk, WTD, or MD provided any protection against CWD infection. All of the genotypes studied experimentally became infected after exposure to an infective dose of brain tissue from CWD infected animals. All of the various genotypes studied in free-ranging populations of cervids contained individuals from each genotype that were infected.

Any CWD "resistance" conferred by the genotypes studied was not complete and was limited only to the progression of the disease (deposition and accumulation of PrP^{CWD} prions in tissues) and the duration of the incubation period between exposure to an infectious dose of prions and the onset of clinical signs and/or death. All of the genotypes studied after sufficient exposure became CWD test positive at some time post exposure. Also, of significant importance, all infected individuals regardless of genotype shed infectious PrP^{CWD} prions in bodily fluids.

So, at this time, selective breeding for all the genotypes identified and studied for "resistance to CWD" will not be helpful in preventing the disease. Elk with genotypes 132LL and 132LM, WTD with genotypes 96SS or 96SG, or MD with genotypes 225FF or 225FS can all become infected if exposed to an adequate dose of CWD prions. Shortly after infection, all will shed prions for life that can infect other animals by direct contact or indirectly into the environment. At some sufficient time period after infection, all the genotypes above can develop neurological signs of CWD and then die of spongiform encephalopathy.

Regrettably at this time, the genes for resistance to CWD infection in cervids that would be analogous the 171RR gene in sheep for resistance to scrapie, have not yet been identified.

DISCUSSION ON DISEASE RESISTANCE

Discussion on Disease Resistance

To provide complete resistance to a disease, infection has to be prevented. There are cases in animal diseases where infection has been effectively eliminated by either the use of efficient vaccines as in Rinderpest, or by genetic selection as was accomplished with scrapie in sheep.

Another strategy in the control (not elimination) of diseases is in the reduction of the risk of transmission of the disease causing agents to susceptible individuals. For example, vaccines for bovine brucellosis in cattle do not prevent infection but do reduce abortions, which are the primary source of infective tissues and possible exposure to other cattle.

In regard to CWD and cervids, the longer incubation periods associated with some genotypes has been commonly referred to as “resistance”. It may be at the individual animal level, those with certain genotypes do internally and physiologically “resist” the progression of the disease, but every single one of the so-called “resistant genotypes” are capable of becoming infected and with sufficient time also becoming clinically affected and finally succumbing to the disease. Simply stated, presently there are clearly no known genotypes of cervids that resist CWD infection.

DISCUSSION ON GENETIC SELECTION

Discussion on Genetic Selection

Genetic selection has been known since Darwin's time, and is well demonstrated both naturally and through selective breeding. There are several fundamental tenets to the process. The first is that natural genetic selection is done at the population level. The second is that the genotype in question must in some fashion present a selective advantage which results for those with that possess particular genotype achieving increased survival and perpetuation of their genes as compared to other genotypes.

Selective breeding has been done for thousands of years at the phenotypic level (before the discovery of genes but which is controlled by genetics) and is responsible for all the domestic livestock and companion animal breeds. Phenotypic selection is actively practiced today in the farmed deer elk industries for antler characteristics. To achieve success with selective breeding in animals, one must have control over which male is allowed to breed with which female.

If the genotype or genotypes in cervids that confer complete resistance to CWD infection are ever discovered and can be identified in individual animals, perhaps then the farmed deer/elk industry could utilize selective breeding to prevent CWD infections in their herds in the same manner as was accomplished with scrapie in sheep. Virtually complete control of which animal is allowed to breed (either naturally or by artificial insemination) with another animal based on the desired genotype would be essential. If the two major obstacles to breeding for resistance to CWD infection (knowing the CWD genes that confer complete resistance, and complete control of breeding) could be overcome, then eradication of CWD in farmed herds could theoretically be achieved.

While selective breeding for CWD resistance might be possible in the farmed deer and elk situations, control of breeding in wild populations of cervids would be extremely difficult, if not impossible, even if the genotypes that confer resistance to CWD infection were known and identified. Breeding in free-ranging animals is highly random. There is no control over which male breeds which female.

Also, in regard to CWD resistance in free-ranging cervids, there are some questions about the selective pressure (advantage vs disadvantage) that the disease places on populations. Since CWD prevalence rates (infection) of cervids that are tested ranges from 30% in some local isolated populations, and is never more than 9% on a state level, the majority of the individuals in cervid populations are never exposed to an infective dose of CWD. The direct cause of death (morality) due to CWD is always much less than the infection rate. The long duration of the incubation period of 17 months to 5 years, allows even animals infected at birth to breed and produce an offspring or several offspring no matter what the dam's genotype may be.

Discussion on Genetic Selection

It clearly has been documented in elk, WTD, and MD that the genotypes that confer increased duration of the incubation period, longer life spans, and therefore the opportunity to produce more offspring, have a very low frequency in populations. Those genotypes that result in shorter duration of incubation periods and the more rapid progression of the disease before the appearance of clinical signs and/or death, have the greater frequency in the populations. These conflicting data on gene frequency is contrary to any possible selective advantage, and that causes some to question the effects of CWD on the distribution of genotypes within a free-ranging cervid population, which in turn brings into question the basic tenet of natural selection – differential reproductive rates.

LIST OF CRITICAL RESEARCH NEEDS REGARDING CWD RESISTANCE

List of Critical Research Needs Regarding CWD Resistance

1

Obviously the first would be to identify the genotypes that would confer complete resistance to CWD infection, but this will depend on several other research needs listed below and the requisite funding.

2

Experimental infections with statically significant numbers of CWD known naïve animals including controls, orally exposed with an inoculum standardized by source, strain, number of PRPCWD prions, and method of exposure. The exposed animals should be paired with controls by gender, age, source of location, and genotype.

3

The shedding of infectious prions should be comprehensively studied in terms of initiation of shedding, duration of shedding, the body fluids in which prions were shed, and most importantly the number of infectious prions shed by which routes.

4

The minimum infectious dose (MID) of infective prions should be established for elk, WTD, and MD.

5

every CWD test positive sample should be run by Western Blot (WB).

6

Suitable biosecure facilities to conduct CWD experiments safely on susceptible cervid species need to be identified.

7

Experiments done on non-cervid species and/or use intracranial injections (IC) or other not natural routes of infection should be avoided as a waste of limited available funding and not generating useful information.

EXECUTIVE SUMMARY AND BRIEF ANSWERS TO THE REQUESTED QUESTIONS

Brief Answers to the Requested Questions

1

Relationships with susceptibility to Chronic Wasting Disease in North American Elk, White-tailed Deer and Mule Deer with specific alleles of the normal cellular prion protein, encoded by the PRNP gene.

Answer: All studied alleles are susceptible to CWD infection.

2

Known incubation periods in natural and experimental situations for all known genotypes of North American Elk White-tailed Deer and Mule Deer.

Answer: Incubation periods established from experimental infections (exposure to clinical signs/death) varied from 17 months to over 5 years.

3

Understanding when the different genotypes and their exposure to CWD versus their time to become infected. Are certain genotypes less susceptible or is their disease progression slower?

Answer: There is no known difference in susceptibility between genotypes. There are differences in the progression of the disease, the distribution of CWD prions into tissues, and duration of the incubation period.

4

Understanding the differences between animals that become clinically CWD positive after shorter exposure times compared to animals with a prolonged incubation period.

Answer: The differences are biochemical and physiological and vary by genotype and individual but the processes in cervids are not well known or understood.

Brief Answers to the Requested Questions

5

Estimated or known frequency of different genotypes of North American Elk , Whitetail Deer and Mule Deer in the domesticated and free-ranging population.

Answer: No brief answer but the known gene frequencies for species and farmed or free ranging are covered in the appropriate sections above. There is no correlation between gene frequency and susceptibility to CWD infection.

6

Known prion shedding that may take place by animals exposed to CWD with prolonged incubation periods. Understanding shedding in different stages of incubation periods.

Answer: Very few studies on shedding of CWD prions have been done. None on duration of shedding, or quantification of prion numbers shed in various bodily fluids. This is listed as a priority research need.

7

How different genotype animals have the opportunity to impact whether a herd becomes CWD positive? Particularly for known CWD positive states and provinces.

Answer: No matter what genotypes are present, if the animals are exposed to an infectious dose of CWD prions, they will: 1. become infected; 2. they will test positive perhaps as early as 90 days post exposure; and 3. when test positives are confirmed, the premise will be quarantined. After exposure and still living, infected animals will shed CWD prions. These shed prions are capable of infecting other susceptible cervids by direct contact or indirectly through environmental contamination.

8

What scientific shortfalls exist in understanding more about CWD resistance and where should future research be focused?

Answer: Again no short answer but they both (shortfalls and research needs) are listed in detail in sections above on page 32.