Re: "Can Science Breed CWD Out of Deer? Not Likely"

This reviewer has now seen and read your article entitled "Can Science Breed CWD Out of Deer? Not Likely". As such, I have significant concerns about the scientific merit of this article, and have formulated my response below in largely the same format that I would use as a reviewer for a peer-reviewed scientific journal. Please see my point-by-point below, along with supporting peer-reviewed scientific references.

Authors' Title: "Can Science Breed CWD Out of Deer? Not Likely"

Reviewer1: What experimental or peer-reviewed scientific evidence do you have to justify this title? In sheep, the majority of the risk for scrapie (i.e., differences in susceptibility), and natural variation in scrapie incubation periods, can be explained by sheep genetics **[Refs 1-2]**. Moreover, while some codons of the sheep prion protein gene (*PRNP*) have large-effects on scrapie risk and incubation period, the corresponding proportion of risk or incubation period that can be explained by the joint effects of a number of other genes (i.e., polygenic effects) in sheep is both moderate and tangible **[Refs 1-2].** This is similar to what occurs with CWD in farmed U.S. white-tailed deer, where the majority of the risk for CWD (i.e., differences in susceptibility) and natural variation in disease progression can be explained by deer genetics, including both *PRNP* and polygenic effects **[Ref 3]**. Interestingly, scrapie may be one plausible origin of CWD, and we already have a successful National Scrapie Eradication Program in sheep that has reduced the prevalence of scrapie by ≥ 85% **[Refs 4-6]**. Notably, the accuracy of the genomic predictions for risk of CWD in farmed U.S. white-tailed deer at CWD-positive facilities (i.e., ≥ 81%) is remarkably similar to the above stated reduction in scrapie prevalence (≥ 85%) by our national program **[Refs 3, 6]**. Likewise, somewhat similar prediction methods seem to work well for both sheep and farmed U.S. white-tailed deer **[Refs 3, 7]**. Therefore, given the scientific parallels illustrated here, I fail to understand how you might scientifically justify your title?

Authors' Comments Regarding Dr. Brightbill's Statement and Skeptical Scientists: "No one has documented a deer surviving CWD, so no one has a truly CWD-resistant deer. How can anyone say they can selectively breed more of something that doesn't exist?" …… "Pie in the sky" …… "Unrealistic Hopes"…… etc.

Reviewer1: What experimental or peer-reviewed scientific evidence do you have to justify these statements? These statements reflect a fundamental flaw in the authors' understanding of CWD infection/pathogenesis (i.e., mechanistically), the role of host (white-tailed deer) genetics in this process, and the established peer-reviewed literature. At least two established mechanisms exist to reduce CWD susceptibility in farmed U.S. white-tailed deer: 1) Selective breeding for advantageous *PRNP* alleles (i.e., like codon 96S) that are known to directly reduce the conversion efficiency of refolding the normal prion protein (PrP^{C}) into the disease-causing form (PrP^{CWD}) **[Refs 3, 8]**; and 2) Simultaneous selective breeding for advantageous polygenic effects which also reduce CWD susceptibility and disease progression **[Ref 3]**. We have data on farmed U.S. white-tailed deer since 2013-2014; with some farms reaching high prevalence (> 60% or 70%). At moderate to high CWD prevalence, it becomes scientifically unreasonable to assume that all white-tailed deer which remain CWD non-detect over a protracted period of time (i.e., for years; with comingling) were simply not exposed, or that the incubation period is totally unpredictable or infinitely long. Therefore, one can test the hypothesis that underlying genetic differences may be explanatory; by first estimating the heritability of differential susceptibility to CWD, and then by using something like genomic prediction with cross validation in a machine learning approach. This is precisely what has been done in a peer-reviewed publication **[Ref 3]**; which further demonstrates that disease progression is also a highly heritable trait, and thus predictable **[Ref 3]**. Genomic prediction (also known as genomic selection) for genetic improvement of crops and livestock (including aquaculture species) for both disease resistance and production traits is not a new discipline. For example, as of today, a search of PubMed for "Genomic Selection" yields 2,397 results, and a similar search for "Genomic Prediction" yields 1,474 results. I suggest the authors familiarize themselves with this peer-reviewed literature since USDA NIFA, and more recently USDA APHIS, fund competitive research proposals for genetic improvement of U.S. crops and livestock via genomic prediction.

Authors Comments on Unrealistic Hopes and Prolonging Problems: "You'll never produce enough genetically manipulated deer to overwhelm breeding in a wild population," he said. "Maybe you could get 40%, 50% or 60% of the less susceptible genotypes out there, but that wouldn't control CWD." …… "I'm not a geneticist, but these CWD-resistant genotypes aren't found frequently in natural populations," Schuler said. "The most CWD-resistant genotypes occur at very low rates, and there's probably a reason for that. Those genotypes might carry traits that cause other population-limiting problems not yet documented." ……

Reviewer1: What experimental or peer-reviewed scientific evidence do you have to justify these statements? No white-tailed deer are "genetically manipulated" or "engineered" for the proposed breeding program. Selective breeding via genomic prediction only identifies the very best genetics that mother nature has already provided; and serves as a tool to enrich for those advantageous additive genetics in a directed breeding program. Unlike these authors, I am a geneticist, and if 40%-60% of the wild deer had most or all possible advantageous (i.e., additive) alleles that reduce susceptibility to CWD, then the prevalence of CWD in those wild populations would be significantly lower overall. However, I am not proposing to replace all wild deer with selectively bred farmed deer (which is only an ideological catalyst for political propaganda in the form of conservation vs agriculture). I have different funded objectives for wild deer. In the U.S., the most "CWD-resistant" single genotype (i.e., the largest-effect region of the farmed white-tailed deer genome) lies in codon 96 (i.e., 96S) of the prion gene, and as of today, with a very large sample size, this allele is common (96S allele frequency = 0.31). Likewise, in a representative sample of 574 wild white-tailed deer from nearly every range-appropriate ecoregion of TX, the same 96S allele is also very common (96S allele frequency = 0.48). Unlike a recent study **[Ref 3]** which properly corrects for the effects of sex, age, geographic region of origin, and allele frequency stratification due to population structure, most prior *PRNP* case-control studies for CWD do not apply these corrections, or even fit the data to a proper inheritance model (or to an inheritance model at all if only allele counting was used). Likewise, the high heritability directly underlying differences in CWD susceptibility is also largely modulated by common genetic variation **[Refs 3, 9-10]**; which is not a new concept in quantitative genetics **[See Ref 9]**, or specific only to deer and CWD **[See Ref 9 and 11 for example]**. As of today, the average minor allele frequency across all natural genetic variation (genome-wide) used for farmed white-tailed deer genomic predictions = 0.33 (i.e., common genetic variation). Our proposed method for selective breeding (genomic prediction) does not rely on intense inbreeding via single-gene selection on rare *PRNP* alleles that are identical by descent. Both rare and common *PRNP* alleles are embedded in highly variable genomic backgrounds which we holistically differentiate via the "additive genetic merits" (i.e., the genomically-estimated breeding values or GEBVs) **[Ref 3]**. A search of PubMed today for "GEBV" produces 291 relevant peer-reviewed results, and the utility of GEBVs is not restricted to farmed populations or farmed species **[Ref 12]**.

Authors Comments on Skeptical Scientists, Unrealistic Hopes, Prolonging Problems: "Schuler said the most anyone has done is identify deer with relatively uncommon genotypes that delay CWD infection and prolong the deer's life." …… "Those deer just take longer to get CWD"……"Selective Breeding could prolong CWD's challenges." …… CWD Resistant Deer acting "squirrely"…….

Reviewer1: What experimental or peer-reviewed scientific evidence do you have to justify these statements? As I point out above, the first statement here is verifiably false, since common genetic variation is significantly associated with differences in CWD susceptibility, and common genetic variation also underlies the high heritability of differences in CWD susceptibility **[Refs 3, 10]**. Several common talking points have emerged in debates between conservation and agricultural groups. One of those talking points is as follows: the deer just take longer to get CWD, and they will just shed more. First, we do see genomic backgrounds in farmed white-tailed deer that have never become CWD-positive at CWD-positive facilities in our current and prior work **[Refs 3, 10]**, and more recent heritability estimates on the liability scale **[Ref 9]** are also high **[Ref 10]**, despite the inclusion of many more nationwide samples. Second, in our selective breeding program, we select on genetic features that are known to directly reduce the conversion efficiency of refolding the normal prion protein (PrP^{C}) into the disease-causing form (PrP^{CWD}) **[Refs 3, 8, 10]**; and deer bred according to our program would not be expected to shed more of something (i.e., PrP^{CWD}) that is created with significantly less efficiency, or not at all. Therefore, how is this approach in farmed white-tailed deer any different from the wildly successful National Scrapie Eradication Program in sheep; where the so-called resistant sheep can still develop classical scrapie **[Refs 13-15]**, albeit at very low prevalence via natural transmissions **[Ref 14]**? This comparative evidence with sheep scrapie is important, because it illustrates that resistance need not be 100% in order to elicit a successful eradication program in farmed livestock **[Ref 6]**. However,

I acknowledge that I cannot control the selection intensity (or breeding consistency) among all those producing farmed deer, or those participating in a selective breeding program. Another common talking point is that a more rare (but statistically advantageous) *PRNP* allele is risky business; because mother nature must disfavor it given its low frequency, and something bad is likely to be associated with these types of more rare alleles. This too is not a responsible scientific inference; particularly since the lower frequency of such alleles can also be easily explained by their origin (i.e., as newer, younger naturally occurring mutations). A deer "acting squirrely" (with n = 1 observation) by a graduate student is both subjective and scientifically meaningless, but it is perhaps mildly effective at negatively influencing the lay public. I've seen many wild deer "act squirrely" (i.e., nervous, erratic, etc).

Authors Comments on Recognizing a Mirage: "It's unlikely we can breed CWD out of deer, news that is disappointing to many deer hunters. But Kip Adams, NDA's Chief Conservation Officer, said it's good when we know early on that a potential solution is only a mirage."
Reviewer1: This reviewer agrees that mirages exist in the present article; he just disputes the origin and composition of those mirages. The first very troubling mirage I see here relates to the content of the article; which purports to be an unbiased scientific opinion piece regarding CWD and selective breeding directed at the general public; yet the article completely disregards all relevant peer-reviewed scientific literature **[i. e., See Refs 1-15]**. Unfortunately, I cannot deduce if this is because the authors are simply operating outside of their areas of expertise, or if this strategy is by design. The second mirage I see relates to diverse scientists actually welcoming scientific advances related to reducing the prevalence of CWD, even if they emerge in alternative (i.e., agricultural) environments, and are most easily applied there. How can any group who expresses concern about CWD logically object to, or endeavor to suppress, management strategies that can reduce the prevalence of CWD in an outdoor environment; particularly since environmental contamination is also additive? Wouldn't a reduction in CWD prevalence in farmed white-tailed deer benefit everyone? I do not dispute the complexity and challenge of controlling CWD in free-ranging populations; but as an unbiased professional scientist, I am greatly disturbed by the many statements in this article which lack scientific merit. I also do not see many high-impact solutions for CWD emerging from the various research groups, with some focusing almost exclusively on surveillance, and perhaps that frustration is the motivation for this article? Luckily, neither the authors or the NDA will need to assume I am an expert, and simply take my word for it; as I have provided peer-reviewed scientific references to justify my point-by-point response to your article. After reviewing your article, my scientific decision must be "Major Revision", or perhaps worse.

Respectfully,

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