Chronic Wasting Disease:
A working hypothesis, the Agent and its Transmission

Observations on TSE Transmissibility
PART IId: CWD Created?

R.A. Forrest

The CWD Foundation, Box 55, South Fork, Colorado 81152

Abstract: (Part IId) Transmissible Spongiform Encephalopathies (TSE) and Chronic Wasting Disease (CWD) are devastating neuropathologic diseases caused by a unique, but unresolved infective agent. Rouge prions or more logically, Spiroplasma bacteria have been implicated as the probable causal agent. Astigmatid mites possess the capability of carrying TSE infectivity. Grain, forage and mold mites have a documented role in harboring and perhaps reproducing the TSE pathogen. Psoroptic mites are found in abundance at facilities known to contain CWD infectivity. Psoroptes ingest lymph and can introduce bacteria-contaminated saliva into oozing, mite-created surficial skin wounds. Observational data suggests two methods of origin for CWD in cervid wildlife: 1) forage or environmental mutation and 2) parasite mutation. Environmental or situational stress induces pathogen mutation. Residual or mutant scrapie infectivity in pastures can be assimilated into forage mites. Mite-infested pasture feed is later harvested and fed to captive cervids. CWD ensues. Alternatively, ecto-parasites (believed to be psoroptic mites or perhaps a common rabbit tick) infest scrapie-diseased sheep. Diseased sheep leave sufficient infective parasitic mites in pastures or facilities, which later contain cervids or other species. Mites feed upon a susceptible species, disseminating the pathogen and CWD ensues. Pathogen-bearing mites could be resident upon a non-affected mammalian host, awaiting contact with a susceptible species. The transference of TSE-infected mites either directly or indirectly via other ruminant species may have been achieved without direct contact or obvious TSE infection. Rabbits play an unknown, but conceivably important role in disease retention or potential transmissibility.

INTRODUCTION

Chronic Wasting Disease (CWD) is a Transmissible Spongiform Encephalopathy (TSE) solely affecting cervidea (deer) species. The causative TSE agent is unknown, but is believed to possess unconventional biological and physical properties. Currently accepted theory suggests that TSE is caused by a proteinaceous infective particle or "prion" (Pruisner, 1982). However, one particularly unusual biological agent, the enigmatic Spiroplasma bacterium, a mycoplasma derived from the Class Mollicutes, has been offered as a more logical causal agent (Forrest, 2002, Part I). While considerable controversy still concerns the nature of the TSE causative agent, spontaneous, genetic and transmissible varieties have been described.

Part I, "A Logical Causative Agent" (Forrest, 2002) demonstrated the fallibility of current testing procedures and that Spiroplasma has the distinction of harmonizing with the most known TSE agent characteristics. In Forrest (2003a) Part IIa, "Novel Vectors" the relationship of insects, Spiroplasma and TSE was examined. Astigmatid grain or storage mites have been implicated in the potential transmission of TSE and Spiroplasmas are likely to be present in infective mites. In Part IIb "The Chronic Conundrum" (Forrest, 2003b) wild and domestic CWD case studies linked puzzling and seemingly random or co-incidental events into a consistent, cohesive picture of disease transmission characteristics. The defined pattern of disease is fully conducive to a feed-introduced Spiroplasma infection. In Part IIc, "Transmissible Postulates" (Forrest, 2003c), potential theories of transmission are explained, suggesting the presence of at least two CWD strains, a residual environmental strain, (CWDe) and a virulent respiratory strain (CWDr). Hay or forage mites can act as a residual reservoir of CWDe disease. Cervids grazing upon infected fields, or ingesting mite-infected hay may obtain sufficient pathogen to result in a CWDe infection. A speculative reservoir of CWDe is expected to be present in the ecto-parasite *Psoroptes cuniculi*. Alternatively, with very close animal contact CWDr may be transmitted.

Spiroplasmas, like all mycoplasmas are parasitic, difficult-to-culture bacteria lacking in cell walls, and are, hence, resistant to most antibiotics. Some *Spiroplasma spp.* seek out mammalian membranes and display an appetite for sterol and phospholipids, with the uncanny ability to metabolize parts of the host's immune system. Importantly, they possess a very diverse shape-shifting character including an intracellular "stealth" mode making detection by microscopy difficult.

Despite over 20 years of research, the origins and mode of CWD transmission are still unknown. While the postulates presented herein are hypothetical, they are grounded upon documented and observable phenomena. Significant investigative effort must be completed prior to firmly establishing a true origin-causal agent-transmission linkage. The following information is presented as background and development of potential working hypotheses as to the origin of its causal agent and the consequential development of Chronic Wasting Disease.

CWD: The Early Years

The exact origin of Chronic Wasting Disease is unknown. Without a "way-back" machine, one can only speculate as to the nature of the initial outbreak. The early progression of the disease is informationally cloudy, rightly so until its specific recognition in captive wild cervidea research herds, followed in turn by recognition in wild animals and still later in domestic farmed animals. One must recognize, without prejudice, that the majority of available data for review has been generated by personnel closely, or even intimately involved with the operation of facilities potentially implicated in the possible creation, and most certainly the spread of the CWD contagion. Observation and conclusion bias is predictably present.

Not illogically, wildlife-oriented research personnel have an innate bias to portray Chronic Wasting Disease as a "natural" occurrence of disease. Whereby disease slowly spreads in the wild, and perhaps commercial farms are implicated in the widespread dissemination of disease. Conversely, commercial cervidea farmers, such as the Author, draw differing conclusions from the same potentially-biased field data, but with an opposing approach; a "creation" of the disease within wild animal disease research facilities and subsequent dissemination in the wild via wildlife transplants and trades. Either proposition may be all or partially correct, or both viewpoints could conceivably be wrong.

The natural "wild" option has been well pursued by Miller (1998, 2000). According to Miller (2000) the current distribution of wild CWD cases suggests that the most plausible scenario was the occurrence some 30 years ago of more or less spontaneous CWD in free-ranging deer of North-central Colorado or southeastern Wyoming, spreading via species contact and normal migration routes to the situational scenario now found today. Such a finding is grounded upon the wider distribution of CWD and a higher prevalence rate in south Wyoming. However, documented observational data seems to belie such a simple and exculpatory source and origin.

Historically, a recurrent chronic emaciating disease was first recognized in the late 1960's in wild mule deer held captive at the Colorado Fish and Game Department facilities and the nearby Colorado State University Wildlife Disease Research Center, both at Ft. Collins, Colorado (Williams and Miller 2002). Williams (1982) places the first case in 1967 based upon research personnel observational data. Deer at these facilities originated from free ranging populations and were maintained with routine animal exchanges from Wyoming Fish and Game facilities at Sybille Canyon, Wyoming and the Denver Wildlife Research Center (DWRC) initially controlled by the Colorado Fish and Game Department (pre-Colorado Division of Wildlife), but later turned over to the U.S. Bureau of Sport Fisheries and Wildlife (USBSFW), a predecessor to the current U.S. Fish and Wildlife Service.

In 1978, Williams (1980) first diagnosed "chronic wasting disease" as a spongiform encephalopathy in Ft. Collins animals. Additional cases were noted shortly thereafter at the Sybille, Wyoming research facility. By 1979, two captive Ft. Collins elk, housed adjacent to diseased captive mule deer were noted with similar maladies. About two years after the 1978 diagnosis of mule deer at Sybille, elk cases began appearing within the Sybille facility (Williams, 1982). Prominently, Williams (1982) noted that the disease is apparently limited to wildlife facilities in Colorado and Wyoming. Williams also critically noted the possible fence line contact with wild animals, and the strong, albeit circumstantial evidence of intra-facility transmission from infected mule deer into the captive elk. Ostensibly, no great effort was expended to mitigate this early acknowledged problem.

Identification of the disease followed in at least two zoological collections, a private Wyoming zoo (found in a Sybille, hand-reared elk), and more notably at the Toronto city zoo, Ontario, Canada. Additionally, suspected cases were thought to have occurred at the

Denver, Colorado city zoo (the likely source of the Toronto animals), but was never confirmed (Williams, 1992). The Denver zoo was the source of CWD-positive animals later found in a South Dakota commercial herd.

CWD was rampant at Ft Collins from 1970 well into the 1980's with over 90% of the animals resident for 2 years eventually developing signs of CWD. By 1981, free ranging elk infected with CWD were found within Rocky Mountain National Park, about 35 miles south and west of Ft. Collins (Spraker, 1997). By 1985, CWD had been found in free ranging mule deer, and sequentially in wild white tailed deer by 1990 (Williams and Miller, 2002). Williams (1992) noted that CWD was found in the wild deer population within approximately 50 kilometers of the Ft Collins facilities, and was found in two wild elk but no deer within 50 kilometers of the Wyoming facility. However, Williams also noted that virtually all the Colorado cases were within 5 kilometers of the Ft Collin's facilities. Most certainly, this is compelling evidence of disease "leakage" dissemination via animal release, escape or other mechanism, if not of disease origination itself. To date, the question still remains whether the disease originated at those facilities or was perhaps brought into those facilities from the wild population.

Critically, one must recognize that disease identification within wild populations is much more problematical due to widely dispersed animals, confusing clinical symptoms, time delayed evidentiary degradation prior to diagnostic evaluation and the presence of scavengers literally consuming the evidence. Miller (2000) speculated that due to surveillance data and epidemiological modeling, CWD might have been present for 30 years or more before being detected. Nonetheless, since the wild and captive animals disease appears to be identical, it is not possible to determined whether CWD arose in captive or free-ranging populations first (Williams, 2002)

Creative Circumstances

Early CWD (pre-1980) occurred in the Ft Collins facilities regardless of the animal source. Both those born in captivity and those caught from the wild at young or old ages developed disease. Unexplained neonatal loses were extraordinarily high (Forrest, 2003b). Captive neonatal cervids were commonly raised on dairy cow colostrum and milk formula in various forms. Commercial milk replacer was used for a few years. Once weaned, feed included fresh hay, grass, dried alfalfa hay and grain mixtures with multivitamin supplements, salt or mineral blocks, fed predominately in dry lot situations, but occasionally with grass pasture access. Early thought hinted that feed did not play any role in CWD transmission. No processed animal protein was noted in past rations, however records prior to 1974 are unavailable.

Initially, the disease only struck mule deer and later was then found in elk. A few white tailed deer were maintained with the mule deer for many years, but did not succumb to CWD until the early 1990's. Bighorn sheep and pronghorn antelope were housed nearby, but they have never displayed any TSE symptoms despite many years of possible exposure. Additionally, moufflon, moufflon-bighorn hybrids, moose, mountain goats and domestic cattle, sheep and goats have been facility residents for at least two years out to

many years (+12 years), yet none with development of disease (Williams, 1992).

Since the animal feed was initially ruled out as a source of infection, concentration eventually turned toward the domestic sheep and goats housed with the captive cervidea as a possible source of disease. Prior to 1974, all these creatures were used in nutritional studies, disease research and even radiation dosage experimentation, sometimes housed separately, sometimes combined. Both sheep and goats are susceptible to scrapie (also a transmissible spongiform encephalopathy), and scattered, unverified verbal reports suggest a possible scrapie presence; however, no neurological signs of scrapie were ever documented.

Importantly though, the presence of domestic scrapie herds at or near the Ft. Collins facilities is nevertheless rather well documented. Between 1947 and 1991 at least three and perhaps four sheep flocks were diagnosed with scrapie in Larimer County, the county seat being Ft. Collins (Wineland, 1993). This is the highest documented concentration of scrapie flocks in the state of Colorado. Unfortunately, individual flock statistics are not readily available.

As portrayed in Forrest (2003a) "Novel Vectors", sheep scrapie infectivity has been confirmed in astigmatid mites found in Icelandic sheep pastures. To review a bit, Wisniewski (1996) collected hay mites from five Icelandic scrapie-confirmed infected farms no longer stocked with animals. After processing and homogenization, several farm-specific inoculums were created and injected into mice. Ten of 71 mice (14%) developed clinical scrapie. Five of the ten positives derived from a single farm. A 200-fold concentration of that farm's mite sample revealed positive staining of abnormal proteinase-K resistant protein (PrP^{res}) via the non-sheep-derived, 3F4 monoclonal antibody. Curiously, the 3F4 antibody should not react with supposedly sheep-derived tissues. As extensive measures were taken to prevent contamination, Wisniewski concluded that Icelandic hay mites were potentially a self-sustaining reservoir for a transmissible spongiform causal agent, although not necessarily a specific sheep scrapie version. Incredibly, the reaction of a non-scrapie-derived antibody to the concentrated homogenate suggests a common, generic TSE agent present in the mites, perhaps an agent bridging between scrapie and other TSEs, but not specifically derived from an ovid (sheep) source.

Rubenstein (1998) using the same Wisniewski samples found that the concentrated sample yielded Western Blot protein bands dissimilar from the typical ME7 or 263K scrapie test strains, which would have been prima facie evidence of lab or field contamination. A harsh Proteinase-K treatment again produced strongly stained tissue with the 3F4 monoclonal antibody. Paradoxically, the sample was not stained by 7G5 hamster PrP^{res} sheep scrapie antibody, further precluding any field or lab scrapie contamination event. Rubenstein concluded that an unknown TSE source or agent, which can replicate in the mites, was present in the farm concentrate.

Carp, (2000) following Rubenstein also used the same mite samples, but explored scrapie incubation periods via genetically engineered mice. Three of four sample results

demonstrated PK-resistant protein bands sufficiently similar to the common ME7 scrapie strain, yet still differing somewhat in passage incubation periods. Extraordinarily and quite radically, a fourth isolate was remarkably aberrant, producing divergent two-banded (verses normal three-banded) PK-resistant proteins, with extended incubation periods, prolonged obesity, sans motor nerve dysfunction, yet much more pronounced post-mortem brain tissue vacoulation. The unusual characteristics of isolate 4 did not match or come even close to any known scrapie strain. Carp concluded that such results could not have resulted from field or lab contamination and perhaps a new and unique TSE strain or agent may be resident in the mites.

Most logically, the Icelandic mite evidence suggests: 1) a non-ovid derived source (Wisniewski, 1996); 2) a potentially replicating, previously unknown non-ovid TSE source or agent (Rubenstein, 1998) and 3) a wholly new and fully unrecognized TSE strain or strain agent possessing radically different PK-resistance characteristics (Carp, 2000). All defined characteristics are strategic and pivotal to the understanding of TSE transmissibility. Conclusively, the potential agent is presumably independent of the source species and may conform, or is perhaps mutated to conform to a new susceptible species into which it has been later introduced. Such a conclusion is antithetic to the notion of proteinaceous infectious particles ("prions"), which by definition are host-sourced. Quite simply put, the agent of TSE is not biologically tied to its host.

Spiroplasmic Ingenuity?

Being bacteria, albeit a parasitic one, Spiroplasma is not fully coupled to any host species. It is quite capable of being independently resident either as residual vectors or as replicating reservoirs of potential infectivity. Forrest (2003a) presented evidence that Spiroplasma is present in most insects and has been confirmed in ticks. As such, it is also likely to be present in their close arachnid cousins, astigmatic mites. As described above, astigmatid Icelandic mites were implicated in carrying TSE infectivity, and to be harboring the infectivity in a form that is not host specific. So could Spiroplasma be that pleiomorphic multiple agent? Could the same agent be culpable for the infection of multiple species?

Certainly, the presence of multiple stains of Spiroplasma bacteria within any one given insect host species is virtually assured. Chastel (1987) isolated Spiroplasma from a number of blood-sucking arthropods, including ticks, horseflies, deerflies and mosquitoes. A total of 23 bacterial strains were isolated from just four species of mosquitoes. Six individual strains were found in one human-feeding (*Aedes spp.)* mosquito from the Northern Alps (Chastel, 1985). Vazeille-Falcoz (1997) isolated three Spiroplasma strains from horse flies (*Diptera: Tabanidae*). An important study by Tully (1995) found eight strains of Spiroplasma from western black-legged ticks (*Ixodes pacificus*) collected in Oregon. Interestingly, each strain consisted of a complete mixture of motile, tightly coiled helical cells, together with small coccoid (spherical) cells with diameters ranging from 300 to 500 nm, as well as straight or branched filamentous forms. Multiple strains are found in multiple forms.

Unquestionably, numerous strains of Spiroplasma are readily available in many diverse hosts. Clearly, each strain possesses sufficient identification criteria to make strain recognition possible. If strain recognition is possible, then most logically, stain characteristics and proclivities are also divergent. Such divergence and multiplicity can then be applied to any one host-species. If the insects can have multiple stains then so can a mammalian host, perhaps, even in greater diversity. If an insect vector or reservoir introduces one Spiroplasma to a mammalian host it most likely introduces multiple Spiroplasma stains. Logically, the greater the number of strains available, the higher the likelihood that a viable mutation is present. As such, mutation could provide a form potentially neurotropic to its newfound host.

Scrapie and CWD: Country Cousins?

Are scrapie and CWD similar? Most assuredly, the marked resemblance of central nervous system lesions, epidemiology and glycoform patterns amongst cervidea species strongly suggests that the CWD agent is essentially the same for farmed, captive or fully wild free ranging deer (white-tail, black tail and mule) and elk (Williams, 2002). While CWD can be passed to several species of cervidea, is it compatible with scrapie, the TSE of sheep?

Race (2002) found that the Western Blot TSE protein patterns from deer, elk, sheep and cattle varied considerably from animal to animal. This, perhaps, reflects a range to be expected from randomly selected, heterogeneous populations of TSE-affected ruminants. Notably, however, animal species patterns clustered closely, but were generally not overlapping and were statistically different from other species, although deer and elk patterns were very similar. Race additionally cites a relatively tight grouping of CWD-affected elk glycoform patterns, compared to that of mule deer, which might indicate that elk are infected with a single strain of CWD agent, whereas, mule deer are infected with multiple or different strains. Alternatively, the different PrP^{res} patterns could represent infection by one or more specific TSE strains, not yet characterized for these species.

Most importantly, the general similarity of PrP^{res} glycoform patterns in scrapie-affected sheep and CWD-affected cervids seems to support the hypothesis that CWD arose from sheep scrapie (Race, 2002). Similarly, in-vitro documentation of the susceptibility of normal sheep prion material to conversion into aberrant abnormal protein-resistant prions in the presence of deer CWD protein-resistant material showed reduced, yet effective conversion despite the expected species barriers (Raymond, 2000).

Under experimental laboratory conditions, scrapie has been transmitted into Rocky Mountain elk (*Cervus elephus nelsoni*) (Hamir, 2003). Six domestically raised 3 to 4 month-old castrated male elk were intracerebrally inoculated with one milliliter of an undetermined scrapie stain homogenate derived from 13 scrapie brains from seven different flocks. One elk died of abscess-induced encephalitis at six weeks post-inoculation, two died of accidents at 6 months and 15 months post-inoculation. Two others developed a mild loss of appetite and minor weigh loss prior to brief neurological aberrations culminating in death at 25 months and 35 months post inoculation. One

injected elk and two controls were still normal after 43 months. Albeit with a small sample size, the clinical symptoms of scrapie in elk were not at all similar to CWD, but the brain biopsies revealed strikingly similar lesions and immunostaining virtually indistinguishable from CWD (Hamir, 2003)

On a curious biological note, Bastian (1987) demonstrated that intraperitoneal and dorsal cervical subcutaneous inoculation of neonatal rats with the vertebrate virulent GT-48 strain of *Spiroplasma mirum* produced alopecia (localized hair loss), cataracts, retinal degeneration and a general reduction in body weight ("runting"). Migration from peripheral tissue into central nervous system indicated that GT-48 was neurotropic. Interestingly, alopecia and puritis (itching) had progressed to the head and shoulders of the injected rats by day 20 of the study (Bastian, PC 2003).

Significantly, subcutaneous injection as performed by Bastian, approximates the postulated Psoroptic mite infection route proposed by Forrest (2003c). Under this mechanism Spiroplasma would be introduced at the abraded skin surface by mite lymph feeding activities, and thereby, is allowed entry into the hosts lymphatic system. Dorsal lymphatic transport of Spiroplasma and temporal residence in the dermis could perhaps explain this spreading rodent alopecia and puritis phenomena. This manifestation, in turn, raises the controversial supposition that sub-dermal Spiroplasma infection, moving along the lymphatic system may produce the prominent, namesake "scrapie" sheep symptoms of localized alopecia and puritis. One can then ask: Is scrapie manifested as a surficial Spiroplasma skin disorder immediately prior to settling into a neurotropic, Spiroplasma-induced spongiform phase? The evidence is enticing, and circumstancially scrapie and CWD are intimately related.

Notably, sheep scrapie has been present in the United States since at least 1947. As documented by Wineland (1993) multiple scrapie-affected flocks were present in northeast Colorado's Larimer County sometime after 1947, most probably in the late 1950's and into the 1960's. In northeast Colorado and southeastern Wyoming, sheep, deer, and elk all share pastures and rangeland. If scrapie-affected sheep were present in mixed species conditions, either on the range or in controlled facilities, and were placed in stressful situations, such as starvation, toxin exposure, weather-related events, etc., then cross-species transmission might and could have occurred. Such man-made stressful events were regularly introduced to animals housed at the Ft. Collins research facilities under the guise of various investigative programs.

Although, by no means unusual, one particularly interesting program was undertaken by Schoonveld (1971), whereby sheep and mule deer were confined together and subjected to malnutrition studies to help explain wild deer die offs. Schoonveld was later quoted that some of the sheep may have had scrapie, which was circumstantially corroborated by CSU personal (Rocky Mountain News, 11/5/2001), but as yet no objective substantiation has been realized.

Essentially, though the movement of sheep in and out of controlled facilities for research programs over many years could have singly or repeatedly introduced scrapie agent to the

facilities, all the time without obvious recognition. The short-term nature of most of the research studies, generally oriented toward Master's degree studies, would have precluded long term retention of such experimental sheep, essentially not providing sufficient time for scrapie symptoms to appear. To date, no suggestive symptoms have been recorded, but the records are not prolific.

Interestingly, a controversial adjunct thought involves the work of Purdey (2001), which focuses on TSE clusters and high elevations. Purdey notes an unexplained geographical characteristic of sporadic TSE clusters, generally of an isolated rural nature, together with their position at high altitudes. He cited examples of human CJD clusters in the mountains of Slovakia, Sardinia, Sicily, Japan, Papua New Guinea and of CWD in deer of the east central Rocky Mountains, as well as, scrapie clusters in sheep in the mountains of Iceland, Spain, United Kingdom, and more recently in Sardinia. Supposedly, the chronic hypoxia of high-altitude living seemingly renders mammals more susceptible to oxidative stress, as well as increasing the permeability of their blood/brain barriers with the natural challenge at higher levels of ultraviolet radiation. Species affected by TSEs involve both wild or domesticated animals, which are often compelled or simply choose to spend a greater part of their daylight hours feeding or resting in unshaded open countryside (Purdey, 2001)

Most certainly the Colorado-Wyoming high CWD prevalence area meets Purdey's prerequisite. As does the conditions found within the Ft Collins research facilities, where contemporaneously confined deer and sheep were housed in open, mostly unshaded pens. Such conditions are certainly not unusual and can be found virtually anywhere on any continent. Conversely, the more recent CWD occurrences, such as those in wooded southern Wisconsin terrain undermine such a premise, unless one simply looks at the initial outbreak of the disease without regard to subsequent regions of disease transmission.

The higher elevations of Colorado-Wyoming area, combined with increased ultraviolet radiation may indeed induce UV-related stress, not only in mammalian species, but also in virtually all species of plants and animals, including insects. Further, it is not inconceivable that ultraviolet radiation may as well affect bacteria; particularly those resident within or on insects found in exposed circumstances, such as open field or skin mites. As such, one could then credibly imagine a greater likelihood of UV exposure and, hence, bacterial mutation within or on such insects. Perchance such bacterial mutations are available to attack unfortunate host animals, perhaps, creating variants of disease not heretofore existent. This leads to the contentious conclusion that perhaps a potential scrapie infectivity agent, such as a Spiroplasma may have "evolved" at the higher elevations so as to "create" chronic wasting disease. Such a mutation could still possess most of the diagnostic characteristics of the original scrapie agent, yet be modified to produce CWD. Certainly, other possible causes of bacterial mutation machinations are equally plausible.

In any event, by whatever means of mutation, the findings of Wisniewski (1996), Rubenstein (1998) and Carp, (2000) demonstrate that the scrapie agent is not host

specific and may, in fact, be present in unexpressed multiple forms or strains. Such strains may evolve or may be ever evolving varieties dependent upon the stress of location, or of stress upon a vector or resident reservoir or, perhaps, even as a function of the stress upon a potential host.

Interestingly, the physical location, climate, elevation, vegetation, etc. can all obviously affect a susceptible insect vector or reservoir. Hence, it can be readily assumed that the actual TSE causal agent, particularly if bacterial, might just possess similar variation. A strong argument can be maintained that each diverse location where CWD is present and has been transmitted via an environmental mechanism may contain a slightly modified or evolved agent adapted to its respective environment. Hopefully, only a smattering of these postulated mutants might retain mammalian neurotropic tendencies.

Postulated CWD Origination

One must view the question of CWD origination as being one of possibilities adjusted by probabilities. We may never know the true origin of CWD, and most likely CWD will remain an epidemiological mystery forever. But certainly any creative individual can imagine an array of possibilities ranging from the virtually absurd to the highly probable. Given that to date, after tens of millions of research dollars, no one has yet defined the causal agent of TSE disease, the probability of Spiroplasma being the long sought after causative agent appears quite credible. If one can reasonably accept Spiroplasma as the probable causal agent of TSE disease then, perhaps, the role of insects in transmission is much more certain. If insect transmission is plausible, then selective deductions can be derived from the cacophony of disease information available.

From the author's bacterially accommodating perspective, the following represents two of the more plausible scenarios for the creation, or initial origin of Chronic Wasting Disease. Albeit, these precepts are speculative based upon a very limited number of concrete facts. The two scenarios described, herein, could have been derived from purely wild animal interaction, but the recognized and documented history of the disease suggests otherwise. Abundant additional work will undoubtedly refine or refute these divinative postulates.

The intimate relationship between research facilities and the recognized CWD prevalence is irrefutable. The virulence and the persistent recurrence of disease within the noted research facilities would certainly suggests a concentrated and prolonged pervasiveness of disease, much more so than then ever found under wild field conditions. Research activities conducted in high altitude, exposed pens and upon closely confined animals, particularly those under going dreadfully stressful testing events such as the documented malnutrition tests, lethal dose pesticide studies, heavy dose radiation studies, et al, provides a prospectively ideal incubation situation for the creation of a new disease. This is particularly true of critically challenged, immuno-deficient animals exposed to a myriad of potentially mutated causal agents.

While a fully natural creation of CWD cannot be eliminated, the collective nature of TSE

disease openly suggests direct human intervention. Other than scrapie and the original sporadic or genetic human CJD, fully the majority of currently recognized TSE diseases are human created or most certainly human influenced, witness: mink encephalopathy, Mad Cow, iatrogenic CJD, feline TSE, etc. Further, one can be confident that wild, open field conditions are not as conducive to concentrated animal disease events, such as for instance, the intense nose to nose transmissibility which seems to be needed for respiratory CWD transmission.

Notably, if CWD had originated in the wild, one would have expected a greater recognition of the disease under the observed field conditions from the late 1960's through the early 1980's. While one cannot second guess direct field operating personnel, and one must recognize that field identification is patently more difficult than that under confined conditions. If present and persistent in the wild, CWD would likely have been identified much earlier in the wild once specific, albeit limited, surveillance had begun in the late 1970's through early 80's. As such, CWD was more probably "created" than naturally produced.

Original Environmental Creation

Creating CWD from scrapie requires one's creative imagination following a plausible progression of events culminating in resultant Chronic Wasting Disease. The most credible creation scenario revolves around the findings of the Iceland scrapie infectivity found in astigmatid forage mites (Wisniewski, 1996, Rubenstein, 1998, and Carp, 2000).

Any original environmental creation event is predicated upon the past presence of scrapie-infected sheep reasonably nearby to facilities eventually housing wild cervids. Larimer County, Colorado has a documented history of scrapie, the most plentiful of all of Colorado. Scrapie-infected sheep most assuredly shed the causal agent, believed to be a mutated or multiple-strain Spiroplasma into pastures and fields via dung or urine, or lesser so by saliva or carcass decay. Astigmatid grain or forage mites or other insects, feeding or rummaging amongst such debris, contact or ingest the covert causative agent. Within the mites the agent reproduces and is readily passed to successive generations. A latent reservoir of disease has then been created housing not only a specific scrapie variety of agent, but perhaps of a commensal or mutant form of a prospective CWD agent.

Eventually the mites, a few containing a deviant scrapie agent, are present in sufficient numbers to: 1) be harvested into commercial hay, or 2) to migrate in sufficient numbers to progressively infest and infect adjacent fields or pens. Nearby, penned wild captive research cervids under close confinement are undergoing immuno stressful experimental studies. Such studies limit their ability to ward off a newly introduced pathogen. The wild captive cervids are fed infected commercial hay or are allowed to graze upon infected mite infested grass or forage. Sufficient mites and infectivity are ingested by one or more immuno-suppressed cervids to allow passage of the radical and resistant new causal agent into the animal's lymphatic system. Incubating and spreading, eventually CWD is manifest. Sooner or later, enough captive, disease-exposed wildlife are passed

through the facility and returned to the wild to establish the disease under wild conditions. See Figure 1 for a diagrammatical portrayal of a theorized progression of events.

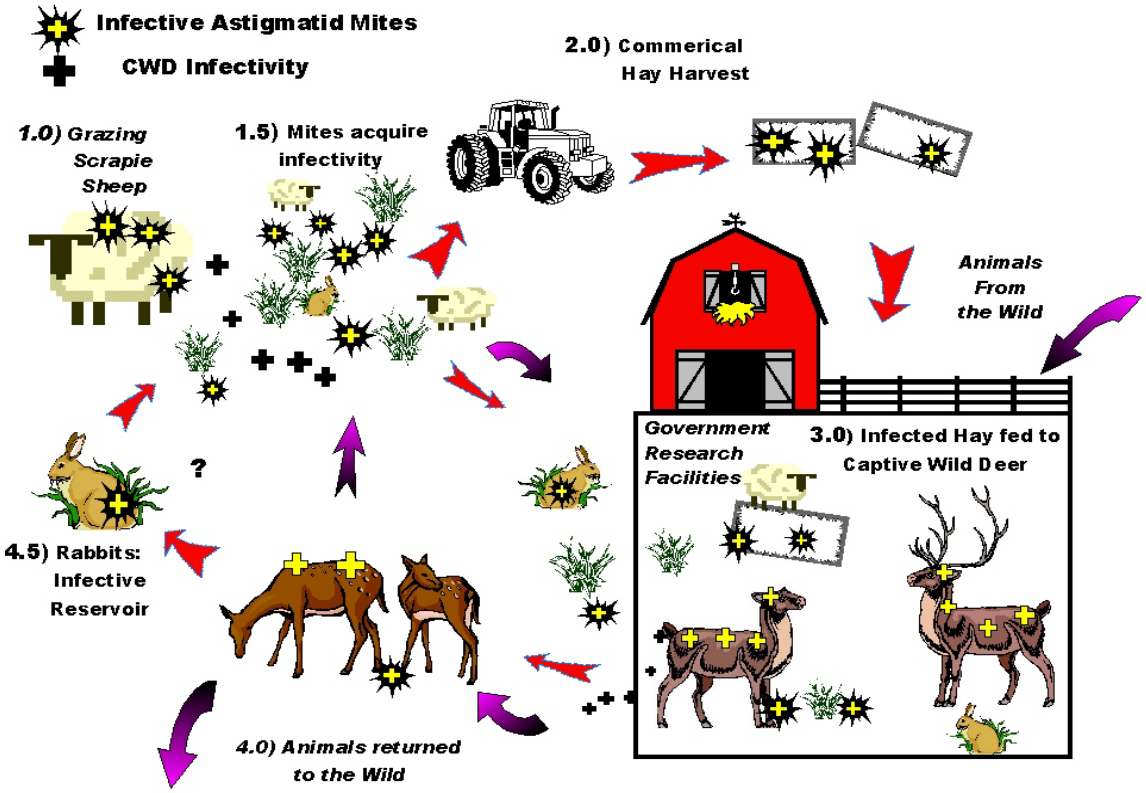

Figure 1: Postulated Original Environmental CWD Infection Scenario.

> ***1.0)*** *Scrapie infected sheep graze nearby to research facilities,* ***1.5)*** *Astigmatid mites, either grass dwelling or ecto-parasites acquire TSE infectivity,* ***2.0)*** *Commercial hay operations harvest infected mites into bales,* ***3.0)*** *Commercial hay is fed to captive wild deer. Spiroplasma from mites overcomes an immuno-suppressed deer's immune response initially creating Chronic Wasting Disease, which can then be spread to others.* ***4.0)*** *Infected deer are returned to the wild, together with incidental infected mite transport to outside of containment pens, hence seeding CWD into the wild cervid population,* ***4.5.)*** *Infected ecto-parasite mites pass onto wild, free ranging rabbits, and the rabbit perhaps becomes a sustaining CWD reservoir in and out of the infected pens.*

Under this scenario the disease would most likely be rampant in the facilities after several years, yet remain relatively delayed and widely diffuse in the wild, mimicking observed conditions. Note that rabbits or other rodents may act as temporary reservoirs or future conduits of disease, assisting in the spread the pathogen amongst numerous field locations or perhaps even directly into the research pens themselves. Once into the wild at large, the disease cycle becomes self-perpetuating and progressively escalating.

However, disease prevalence remains greatest in pens facilities, and secondarily so in locations where exposed wild animals have migrated or been relocated.

Alternative Original Ecto-parasite Creation

As an alternative to the feed-introduced postulate, one must also look at the quite possible, but wholly hypothetical role of ecto-parasites in the potential creation and transmission of TSE and CWD. As demonstrated in Forrest (2003c) "Transmissible Postulates", parasitic body-dwelling insects of the Acari family, namely mites and ticks may play a much greater role in TSE disease transmission then currently recognized.

Psoroptic Mite

As required for the feed introduction route, the presence of scrapie-infected sheep in pastures is again crucial to instigate a series of events culminating in CWD. While scrapie sheep shed the pathogen into the environment they also provide sanctuary for a myriad of potential ecto-parasites. The psoroptic mite, as well as various varies of ticks make use of their unwitting host by consuming lymph or blood. In particular, the lymph-sucking nature of Psoroptes makes it an ideal vector for the inter-animal spread of potential lymph-borne disease.

Recalling the TSE infectivity data of Forrest (2002), lymph nodes are important sites of initial TSE discrimination through the recognition of abnormal prion protein (Sigurdson, 1999). A large proportion (98.4%) of blood-borne infectivity was recovered from the mononuclear leukocyte fraction (Holada, 2002), and leukocytes from a TSE-infected human blood were found to infective (Tamai, 1992). Even B-leukocytes are thought to assist in the transport of scrapie infectivity (Klein, 1998). So fundamentally, *Psoroptes* and lesser so the tick species, derive their sustenance from a highly infective TSE-agent-bearing substance. Forrest (2002) suspects that Spiroplasma bacteria, using the white blood cell rich lymph, have an innate ability to confound the host's immune system and thence gain access to the central nervous system creating TSE disease.

Fundamentally, the postulated parasite-borne route involves the contamination of psoroptic mites through their ingestion of the TSE pathogen derived from scrapie sheep lymph. Infected mites are passed from sheep to sheep until at some stage infected sheep are introduced into the government research facilities. Close bodily contact with cervids virtually assures passage of mites from animal to animal, although a more likely scenario involves cervids occupying contaminated sheep facilities shortly after use by sheep. The mite reproduction cycle allows for several weeks survival off of a primary host, awaiting a new potential host. Unfortunate cervids are those potential new hosts.

Alternatively, scrapie-mite infested sheep are housed with other mite susceptible ruminant species, such as cattle or bighorn sheep. Contaminated mites reproduce and may even perhaps inhabit or non-clinically infect these non-scrapie, or non-CWD-susceptible species. These non-affected individuals then provide a reservoir for future

prospective introduction into captive cervids when placed under close confinement or perhaps utilizing the same facilities. Eventually wild captive cervids passing through the facility will introduce disease to the wild. This second scenario again produces rampant confined facility disease, but with lagging and diffuse disease in the wild.

Conversely, contaminated mites can proliferate upon a scrapie-infected sheep until sufficient numbers are available, allowing for drop off or scraping off of mites onto scratching posts, bedding or perhaps into tall forage. A wild cervid could perhaps utilize the same bedding or scraping feature and, hence, obtain a direct mite infestation or subsequent CWD infection derived from a more "natural" infection route. That unfortunate animal is then subsequently captured and is trans-located into a research facility thereby enabling the spread of the pathogenic mites to other captive cervids. Figure 2 diagrammatically portrays of a theorized ecto-parasite progression of events.

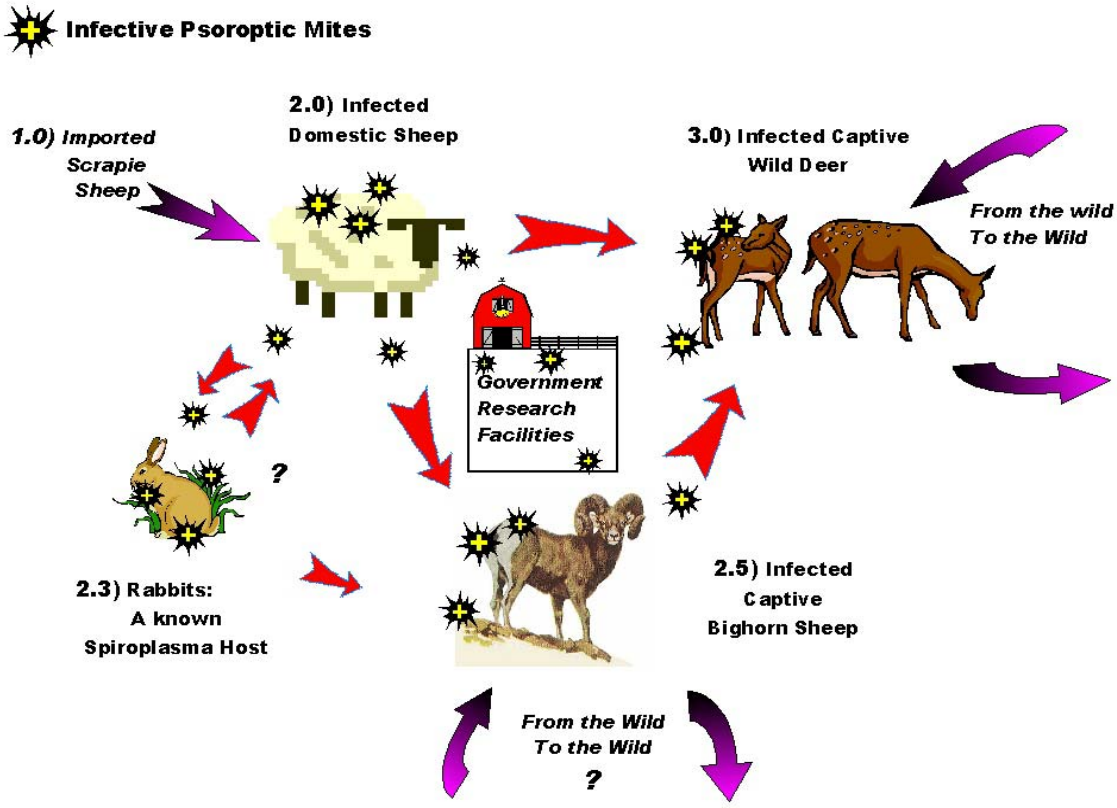

Figure 2: Postulated Original Ecto-parasite CWD Infection Scenario.

> ***1.0)*** *Scrapie infected sheep are imported into government research facility,* ***2.0)*** *Psoroptic mites eat tissue and suck lymph from the infected host, becoming infected with a scrapie variety of Spiroplasma. A mite-infested scrapie sheep makes bodily animal contact or itches upon commonly used man-made facilities, depositing infected mites.* ***2.3.)*** *Infected mites pass onto wild, free ranging rabbits, a known Spiroplasma*

> *host, and the rabbit becomes a sustaining CWD reservoir;* ***2.5****) Infected mites pass onto captive Bighorn Sheep or other* ***NON-susceptible*** *species while kept in close research confinement, creating other possible non-affected host reservoirs,* ***3.0)*** *Captive wild deer acquire intense levels of infected mites, decreasing immune defenses while having scrapie Spiroplasma injected into lymph. Spiroplasma eventually overcomes the deer's diminished immune resistance creating Chronic Wasting Disease. Infected deer, or other species are returned to the wild, together with incidental mite transport to outside of containment pens, hence seeding CWD into the wild cervid population.*

However, under this more wild scenario, the disease would eventually be rampant in the facilities after many years, but wild disease proliferation would be greater earlier in the disease progression, a concept not compatible with the wild observed conditions.

Again, as stated previously, the role of rabbits is an unknown but a suspiciously important wild card. Could wild or even domestic rabbits placed in contact with scrapie-mite infested sheep, or making use of structures or bedding utilized by infested scrapie sheep, become intermediate carriers of diseased mites, perhaps even possessing a non-clinical form of disease? Certainly the presence of the *Spiroplasma mirum* strain in rabbit-borne ticks (Tully, 1983) gives one reason to pause, particularly as *S. mirum* is seemingly responsible for experimental rodent spongiform encephalopathy (Bastian, 1984). The role of rabbits passing mites or the pathogenic agent itself directly to cervids or to other possible carriers is virtually an uncultivated concept.

A continuing follow-on paper: **Chronic Wasting Disease -** Part IIe **Observations on TSE Transmissibility –"CWD - on the move"** will explore where CWD might now be present and various strategies to mitigate potential problems.

The CWD Foundation: T*he CWD Foundation is dedicated to uncovering the mysteries of Chronic Wasting Disease. Fundamentally, the cervid species present a unique opportunity to study natural TSE disease. Cervids are the only unmanipulated gene pool of TSE-bearing species available for experimental research. Once the agent is identified and treated in deer, other species and eventually humans will benefit from the results learned. While it is our belief that cervidea are the solution species to all TSE problems. your assistance is needed. Each donated dollar helps pay phone bills, travel costs, printing costs, etc. A much larger effort is needed. Detailed insect collection, field interviews, lab mice studies, and bacterial PCR-DNA analysis all cost money. A vaccine seems possible but will be expensive in time and money. The required research funds are unlikely to come from official government sources, as the concepts presented herein are not mainstream, and perhaps regarded as unworthy of funding. But after absorbing these studies, perchance one can see the logic for a simpler explanation of CWD. We welcome financial support from concerned parties. Contact: Rich Forrest or Jan Elsworth, The CWD Foundation, Box 55, South Fork, CO 81154, telephone: 719-657-0942, email: Research@uselk.com*

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