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

Observations on TSE Transmissibility
PART IIb: The Chronic Conundrum

R.A. Forrest

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

Abstract: (Part IIb) Transmissible Spongiform Encephalopathies (TSE) and in particular, Chronic Wasting Disease, are devastating, degenerative central nervous system disorders caused by an unidentified, but unique infective agent with a strong resistance to normal disinfectant and sterilization procedures. Several attempts at eliminating scrapie and CWD from infected facilities have been unsuccessful. Captive wild cervids held at the two Colorado government facilities and a third Federal facility have experienced high neonatal mortality rates exceeding 60%. One well-documented facility also had a 79% CWD death rate among cervids surviving to adulthood. Ranch-raised cervid CWD infections have randomly occurred in the northeast Colorado endemic area, seemingly with only their location and source of feed as a common link. In 2001, a non-endemic area elk ranch had a late-stage, CWD-positive mother elk deliver a clinically abnormal calf. The atypical calf possessed a unilateral cataract and a continuous nasal discharge. Within seven days virtually all elk calves born thereafter acquired the same agent generally resulting in septicemic mortality. Ranch mortality paralleledthe neonatal mortality rates of CWD-contaminated government pens. Past research work has determined that a Spiroplasma bacterial infection was capable of producing cataracts, septicemia and other neonatal compatible symptoms. Spiroplasmas are capable of producing spongiform encephalopathy in lesser rodents. Vertebrate pathogenic Spiroplasma varieties are commonly found in insects particularly of the phylogenic Order of Acari (ticks and mites). TSE infectivity has been found to inhabit astigmatic mites common in pastures. Wild, infective CWD cervids can potentially contaminate pastures and commerical hay fields with a feces- or urine-shed causal agent. The agent can be picked up by insects, and transferred via grazing contact with susceptible cervids. Contaminated insects could be transported in baled forage thereby explaining the sporadic occurrences in domestic cervids, as well as the CWD outbreak now found in New Mexico wild deer.

INTRODUCTION

Chronic Wasting Disease (CWD) is a Transmissible Spongiform Encephalopathy (TSE) affecting both wild and domestic cervidea. A proteinaceous infective particle (abnormal "Prion" or PrP^{res}) has been postulated as the suspected disease pathogen (Prusiner, 1982),

however, other more conventional agents, such as a Spiroplasma bacteria have been offered (Forrest, 2002, Part I). Considerable controversy concerns the nature of the TSE causative agent, some are spontaneously induced, some under genetic control and still others, like CWD are readily transmissible. TSEs are distinguished by unusually long incubation periods with progressive central nervous system degeneration and characteristic histopathological lesions. TSE victims lack a significant immune or inflammatory response; while possessing unconventional biological and physical etiologic properties.

In Forrest (Part I, 2002) evidence demonstrated that Spiroplasma, a mycoplasma derived from the Class Mollicutes, retains the distinction of harmonizing with the most known TSE agent characteristics. Spiroplasmas, like all mycoplasmas are parasitic, fastidious bacteria lacking in cell walls. They have an affinity for mammalian membranes, and a specific appetite for sterol and phospholipids. Further they are capable of producing highly oxidant hydrogen peroxide and will invade, occupy and metabolize parts of the host's immune system. Importantly, they possess a shape-shifting character with the ability to enter an intracellular "stealth" mode making detection difficult.

Diverse varieties of Spiroplasma have demonstrated plant pathogenic characteristics while others are capable of producing neuropathic conditions in vertebrates. Inoculation with tick-derived Spiroplasma species has revealed a strong vertebrate neuro-pathogenicity. Part IIa (Forrest 2003) determined that insects might play a very important role in TSE transmission.

THE CHRONIC CONUNDRUM

CWD has displayed extremely curious epidemiological characteristics over its 35-year recognized presence. Several studies have documented the outbreak and spread of CWD in government-operated research facilities of Colorado and Wyoming (Williams and Young, 1980,1982, 1992a&b). Significantly, the CWD disease progression somewhat mimics the epidemiological peculiarities of other TSE diseases, predominantly those of scrapie another more common ruminant TSE. Over several decades of TSE study, a particularly disturbing epidemiological phenomenon has consistently reappeared within several TSE diseases, specifically those of pasture-based ruminants. The chronic conundrum is the apparently inevitable infection of a documented TSE-free ruminant flock or herd when placed upon the premises of previously depopulated, TSE-infected ruminant herd. Re-appearance occurs after months of animal-free, time-resting and perhaps even after full hypochlorite disinfection of the exposed facilities.

TSE DISEASE ERADICATION ATTEMPTS

Palsson (1979) documents an Icelandic scrapie control effort, which removed sheep for up to three years from infected pastures. When scrapie free flocks were re-introduced, scrapie reappeared after a few years. In one particular Icelandic farm, sheep flocks have been eradicated three times, and each time the farm left without sheep for two years and

after restocking with scrapie-free sheep, the disease reappeared.

Eradication of CWD was attempted at the Wyoming Fish and Game research facility at Sybille Canyon, as was documented in Williams and Young (1992a). All deer and elk held in an area of the facility affected by CWD were killed and the pens left vacant for one year. Other ruminants (antelope, bighorn sheep, moose) and apparently other elk and deer in non-affected portions of the facility were not removed. New animals, derived from various locations where CWD had not been recognized, were later placed in the vacant pens, but the first case of recurrent CWD appeared approximately five years later. Either contaminated premises or the shedding of infectious agent by clinically unaffected species was speculatively suggested.

A similar experience noted by Miller (1998) using Rocky Mountain elk affected by CWD. In 1985 all cervids residing at the Colorado Division of Wildlife's Foothill Wildlife Research Facility ("FWRF") were killed. Paddocks were treated with 1,000 PPM calcium hypochlorite solution, then plowed to a depth of one-foot and then re-sprayed with hypochlorite. All feeding and watering equipment was disinfected and a second game fence added to the perimeter. Cervid paddocks were physically separated by spatial buffers and then left fallow for 12 months. Twelve wild captured elk calves were then later introduced from Rocky Mountain National Park, ("RMNP", unfortunately where low-incidence CWD had been identified in the wild in 1981) together with other animals from outside the CWD endemic area. Animals developing disease symptoms were immediately isolated and then killed once a clinical presumption of CWD was made. The first case reappeared at 39 months (September, 1989) after reintroduction, some 51 months after removal of infected animals. The initial case was followed by additional cases in the original 12 calves in May 1991, June 1992 and February 1995 (53, 66, and 98 months respectively after reintroduction). Regrettably, these tame animals were used in cattle-elk field grazing studies on the Colorado Western Slope and may have significantly contributed to the CWD problem recently recognized in Western Colorado (Steele, 2003).

Remarkably, there are numerous documented cases of TSE disease recurrence after removal of infected ruminants. The disease has seemingly "appeared" or reappeared spontaneously without an obvious epidemiological track. One can only draw a limited number of conclusions, either CWD: 1) has an incredibly environmentally persistent etiologic agent, or 2) has a residual presence held in a perpetuating, albeit latent, disease reservoir, or 3) is sustained by an undetermined, perhaps non-clinical vector or potentially a secondary, unrecognized animal host. An examination of case studies may be helpful.

CWD CASES IN CAPTIVE/WILD CERVIDS

Expanding upon the elk calf study described above, Miller (1998) studying all elk CWD cases at the FWRF determined that compelling evidence of lateral verses vertical transmission was present in elk with an average period of 21 to 24 months between cases (range 13 to 32 months) while the disease incubation period ranged from 18 to 36 months

(average 26 months). A single point source (perhaps an initially imported RMNP calf) could not plausibly be the source of all CWD since the incubation periods were not consistent. A sequential lateral infection of clinically symptomatic animals passing CWD to herd cohorts was deemed the best fit for epidemiological progression. To explain the initial occurrence, Miller further suggested that environmental contamination or some unrecognized disease reservoir might be present at FWRF. Unfortunately, the majority of detailed animal treatment records and animal dispositions were reportedly destroyed in the Ft. Collins, Spring Creek Flood of 1996.

Interestingly, in Miller (1998), the first three cases occurred among the three smallest 1986 herd cohorts. But, also of interest was that two calves born in 1991, whose mothers eventually developed CWD, had remained clinically normal through mid-1997. Also of note, during early studies on CWD, Williams and Young (1982) noted recurring infections of both hand-reared and captive wild animals at research facilities in both Wyoming and Colorado. Interestingly, The CWD incident rate among hand-raised elk in Colorado approached 50.0% while those of the wild-caught Wyoming adult elk only aggregated 3.3%.

During the mid-1970's, both the Colorado Division of Wildlife (CDOW) facilities at Ft. Collins, and the nearby leased Colorado State University Wildlife Disease Research Center (WDRC) experienced extraordinarily high fawn death rates in both deer and elk. Trindle (1978) noted that over a 7-year period in the mid-1970s the CSU hand-reared mule deer fawn survival rate averaged only 46% with mortality rates as high of 92% in any one year. Most FWRC fawn losses were attributed to enteritis sometimes with bloody yellow-white diarrhea and dehydration (Neil, P. PC 2003), and although the clinical signs were always quite similar, no known pathogenic agents were found despite dozens of routine necropsies. The anticipated E-coli or a Rota-Corona virus was usually not identified. Eventually, CSU researchers came to believe that fawn death loss could be attributed to stress-related adrenal corticoid-induced immuno-suppression, taken advantage of by opportunistic bacterial infection. This was born out by necropsy showing mild enteritis and diminished thymic glands but no detectable biological agent (Trindle, 1978). .

Halford (1974) documents the rearing of 18 mule deer fawns in 1973 at CSU (WDRC), nine hand-raised and nine dam-raised. Three of the nine hand-raised died as neo-natal fawns supposedly with navel ill, and six of the nine dam-raised fawns died as neo-natals of necro-bacillosis. Cumulatively, the fawn mortality rate was 50%.

Also, during this year, Gill (1974) documents the loss of 31 out of 57 fawns (54%) of the CDOW 1973 fawn crop initially kept at a US Forest Service deer facility, termed the Fraser Experimental Forest near Winter Park, Colorado with subsequent transfer to the FWRF. Fawns died at the age of 2 to 3 weeks and death loss was indirectly attributed to pneumonia due to cold and wet conditions. In Neil (1977) despite improved diet and colostrum intake, only 7 of 40 fawns born at the FWRF in 1976 survived to weaning (82.5% mortality) with diarrhea and pneumonia as the suspected cause. Necropsy noted

virtually complete atrophy of thymic glands in many animals.

Significantly, during the period of 1974 through 1979 some 67 adult mule deer were housed at the FWRF. Sixty deer or approximately 90% were bottle-fed, tame animals. Fully 53 or 79% of adult mule deer housed at the FWRF died or were affected by CWD complications (Williams and Young, 1980). The large majority of these CWD-affected adults were the surviving hand-reared tame fawns remaining from the preceding periods of severe fawn mortality. The only other significant cause of death was physical accident.

Mule Deer Fawn Mortality 1968 to 1976
At the Foothills Wildlife Research Facility
Operated by the Colorado Division of Wildlife

Birthing Year	No. of Fawns Hand-raised	No. of Fawns Dead Prior to Weaning*	Percent Mortality
1968	15	6	40.0%
1969	46	40	87.0%
1970	22	2	9.1%
1971 & 1972	0	0	No fawns raised
1973	57	31	54.4%
1974	35	22	62.9%
1975	36	18	50.0%
1976	40	33	82.5%
1968 to 1976	**251 Fawns**	**147 Died**	**60.6% Mortality**

** Excluding fawn mortality in the first 24 hours after birth*

Similar mortality experiences occurred with elk calves as well. Hobbs (1977) documents the 100% mortality of calves brought to the FWRF from the Wyoming Sybille research station in May of 1976. While four of ten calves (seven from Sybille and three from the Denver Zoo) died in June, 1976 (40% mortality).

An attempt to mitigate fawn losses in the CSU pens was documented by Kramer (1971). In the early summer of 1969, the Colorado Game, Fish and Parks Division (now the CDOW) et al., provided 10 fawns, two to three weeks of age. Fawns were isolated in pens 6 feet apart and fed a special, pasteurized bovine milk formula. Within seven to 14 days of arrival, all fawns had contracted diarrhea. At the onset of diarrhea intensive drug therapy was instigated on six fawns using sodium-sulfachlorpyridazine, chloramphenicol and oxytetracycline. Within 1 to 12 days (ave 7.5 days) of diarrhea all fawns had died, irrespective of drug therapy. Although three serogroups of E-coli were identified, none

individually was a common cause of death. All E-coli serogroups were sensitive to chloramphenicol. Despite the obvious presence of E-coli in the rearing pens, six neo-natal antelope fawns reared close by had no disease problems. The author was at a loss to adequately explain the severe mortality in deer fawns yet no problems in the antelope fawns.

Interestingly, a similar mortality experience occurred at the Denver Wildlife Research Center (DWRC) in the late 1960's. The Colorado Fish and Game Department, the immediate predecessor to the Colorado Division of Wildlife (CDOW) constructed the DWRC's large animal enclosures in approximately 1956. The facility was situated on just a few acres located within the Denver Federal Center, Lakewood, CO. and initially contained domestic sheep, wild bighorn sheep, hybrid domestic-Bighorn sheep and wild-caught mule deer (Pillmore, R.E., PC, 2003). After a few years, the operations were turned over to the U.S. Bureau of Sport Fisheries and Wildlife (USBSFW), a predecessor to the current U.S. Fish and Wildlife Service. The USBSFW maintained a mule deer herd of up to 92 animals in three pens on about 10 acres between 1961 and through the mid-1970's (Robinette, 1973). The deer were sporadically used in lethal-dose pesticide studies. The DWRC obtained wild caught adults from the CDOW (likely some of the FWRF fawn mothers) and some tame bottle-fed fawns, also from the CDOW's Foothill Wildlife Research Facility. Surplus deer were returned to the CSU deer research facility (WDRC) or donated to zoos. Detailed animal records have not been located.

In 1967, 1968, and 1969 the DWRC experienced fawn mortality rates of 43, 41, and 8 percent respectively among fawns two to eight weeks of age. In one group, of the 36 fawns with noticeable symptoms, 28 died, 61% of which were males. The suspected cause of death was listed as necrotic stomatitis (oral mucosa infection) or microbacillosis suspected to be caused by *Spherophorus necrophorus*, more commonly known as the foot-rot bacteria. (Robinette, 1973). This is an interesting correlation with the findings of Halford (1974) at CSU (WDRC), which had received animals from the DWRC.

In the wild animal population, the situation is entirely more problematic, and much more difficult to decipher. The recent (Fall 2002) wide-spread, albeit low-incidence rate outbreak of CWD in both mule deer and elk in the northwestern portion of Colorado, well to the west of the previously established northeast Colorado CWD "Endemic" area is an important clue in the transmission of CWD. As of January 8, 2003, as reported by the Colorado Division of Wildlife website, some 23 deer and 20 elk from the western slope of Colorado have been harvested and diagnosed with CWD.
(see http://wildlife.state.co.us/CWD/research_movements.pdf and,
http://wildlifes.state.co.us/CWD/Detected_CWDMap.jpg, February 2003). Recently a Utah mule deer near the western Colorado border has been diagnosed with CWD (UT Div of Wildlife Res. 2/18/03 press release).

Over a ten-year period, on and off from 1975 to 1985, potentially CWD-infected animals were housed at a Colorado Division of Wildlife's Kremmling, Colorado research facility in

Grand County of north central Colorado. Wild pregnant does were captured, calved out and returned to the wild. The bottle-raised deer were then used in grazing studies in the Middle Park area and were retained at the contaminated FWRF during the winters. After over 15 years and no reported CWD cases, despite focused disease sampling activity, in just the short hunting season of late 2002, 14 cases of CWD were reported within 25 miles of the Kremmling facility. The infected animals were harvested predominately along the geomorphologic trend paralleling the Colorado River. This corridor is an obvious lush feeding area and an easy migration route. Along this route tagged animals are known to migrate to and from the Estes Park east slope CWD endemic area several dozen miles to the north and east, across the Continental divide (CDOW, Federal Aid Rept. 1976).

Further evidence is found in far northwestern Colorado at the Little Snake River Research pens where the CDOW conducted cattle-elk grazing studies using Rocky Mountain National Park captured elk calves reared at the FWRF, as mentioned above. These potentially infected tame elk were allowed to graze in adjacent pens with nose-to-nose contact with wild animals for two, 4.5-month periods in 1989 and 1990. Additionally, the wild elk grazed upon areas where the infected tame elk had previous been retained for short periods. Now some 12 years later, 28 documented deer and elk CWD cases have been found during 2002, with at least 18 cases located along known radio-collar elk migration routes from the Little Snake River Research pens across a triangular area (Steele, 2003).

In June of 2002, a single case of CWD in a desert mule deer was reported on the White Sands Missile Range of southern New Mexico (NMF&G Press Release, 6/19/02). This case was about 28 miles north of the San Andres Wildlife Refuge (SAWF) situated entirely within the White Sands Range, where bighorn sheep populations have dwindled due to psoroptic scabie mite infection and mountain lion depredation. In February 2003 five additional cases were announced virtually straddling the SAWF and southwesterly into the western slope of the Organ Mountains east of Las Cruces (NMF&G Press Releases, 2/4/03 & 2/14/03). Hunters and selective NMFG personnel had sampled only 26 wild deer. No captive or domestic cervid facilities are nearby. While suggestions of transported Colorado infected carcasses as feed for the mountain lions has been postulated, no obvious source of the New Mexico disease is known.

DOMESTIC CERVID CWD CASES

Ranch B: NE Weld County, Colorado

For domestic cervidae, an important Colorado and national event occurred with the discovery in August 1999 of a single case of CWD in a 1998 sub-clinical elk heifer at "Ranch B" of eastern Weld County, Colorado. This case later escalated into several score of cases and eventually led to the depopulation of animals on dozens of ranches in Colorado and on trace-outs to 7 states. Formerly, Ranch B had been implicated earlier in CWD trace-backs of a young elk bull which left Ranch B for Nebraska in December of

1995 and was diagnosed with CWD 28 months later in April of 1998, plus a second case involving an older elk female which left the ranch in March of 1997 which died in April of 1999, about 25 months later, at about 6 years of age. A second Ranch B cow had died at the second recipient ranch under suspicious circumstances in July of 1997 three months after leaving Ranch B, but no CWD brain test was done. In mid-June 1999 Ranch B was quarantined to perform epidemiology, record reconciliation and appropriate notification, but had been ironically released from quarantine in mid-July, 1999 with no problems noted.

With the discovery of the subclinical August 1999 animal, found dead the day after a stressful vaccination day, Ranch B was placed under quarantine again pending epidemiological investigation. Thirty-four close pen mates were subsequently destroyed without another CWD case and Ranch B was again released from quarantine early in 2000. With the death of still another CWD positive, Ranch B-raised 1999 heifer, this time at Ranch A in August of 2001, Ranch B was placed in a terminal quarantine which eventually resulted in the depopulation of several hundred animals in February of 2002. Upon Ranch B's depopulation, at least four-dozen positives or suspects were detected, not counting six trace-out positives. Of the approximately 48 diagnosed or suspected cases at least 17 were homegrown 1999 calves and 15 were year 2000 calves. Importantly, thirty-two head or about 67% were likely infected as calves. Interestingly, both calf crops split approximately 67% male and 33% female in each year. Three cases were in ranch-raised 1998 calves. The remaining positive were composed of older bulls and cows of various ages and diverse sources. Ranch B was initially constructed in 1994 in an area later found to be within the expanded northeast Colorado endemic area. The source of feed was widely diversified but included hay from the South Platte River drainage and portions of southwestern Nebraska where CWD was later found in wild deer. Locally grown corn and oats, as well as local and imported mineral pellet supplement were used often. A more detailed account of Ranch B epidemiology remains for a future study.

Ranch A: Rio Grande County, Colorado

At the Author's ranch (Ranch A), a single clinical 1999 elk female (cow #1) derived from Ranch B, died of CWD complications in late August 2001 at the age of 27 months. She was born in June of 1999 and was transported to Ranch A from Ranch B in early April 2000 at 10 months of age together with sixteen other females of similar age. One other of the sixteen herd cohorts suspiciously died of acute chronic fibrinopurulent pleuropneumonia or aspiration pneumonia at 16 months of age, well in advance of the CWD-infected cow, but the mandatory brain submission showed no CWD symptoms. Detailed necropsy detected the unusual presence of mycotic myocarditis, suggesting immuno-suppression. Through the fall and winter of 2000-2001 cow #1 remained rather small, never exceeding 430 pounds (the herd runt), but became pregnant and remained clinically normal until a failure to gain weight was noted in late pregnancy. At about 23 months of age, obvious weight loss was noted and she calved a clinically abnormal calf in early June 2001. As per veterinary necropsy, the calf (animal #1c) died of maternal-

neglect and malnourishment at the age of 37 days. Calf#1c will be discussed herein below. The infected cow displayed clinical signs of typical, progressive CWD. Symptoms included, herd avoidance, lethargy, head drooping, coarse unshed hair coat, excessive thirst, lack of appetite, yet the infected cow regularly positioned herself prone on her chest near a hay bale seemingly desirous of feed. The author noted emaciation with time, and excessive drooling for the last few days with a glossy eye gaze, but with no noted abnormal defecation problems. Her death was observed, she was beheaded for brain sampling and her carcass was immediately removed from the paddock and placed in a burial pit later to be exhumed by USDA personnel.

Approximately 5 months later (February 2002) Ranch A was fully depopulated (over 420 animals including trace-outs) under the USDA CWD Indemnity program. Two additional positives were noted both still resident at Ranch A. Those positives were a 1992 championship breeder bull (Bull #2), which in the spring of 2001 had achieved a Southwest Regional Mature velvet growth record of 38.2 pounds and had successfully bred some 40 cows in the fall of 2001. This bull was brought to Ranch A from source Ranch B in August of 2000, hence was resident at Ranch A for some 19 months prior to depopulation. By January 2002 Bull #2 had not regained weight lost during the fall rutting season (September 11th through November 15th) and was noticeably thin but not emaciated and showed no other obvious clinical signs of CWD at the time of his depopulation in early February 2002. As such, Bull #2 was likely infected at Ranch B. He had multiple brain biopsies revealing well-developed CWD in the dorsal motor nucleus of vagus nerve, while additional positive tissue was found in tonsil and retropharyngeal lymph node (but not in the hypothalamus) confirming advanced stages of CWD. A second, ranch-born 1999 female (Cow #3) was also deemed positive via a single brain sample reported showing advanced CWD in the dorsal motor nucleus of the vagus nerve, but nothing in the hypothalamus, tonsil or retropharyngeal lymph node. She was clinically normal, albeit a bit mousy and small in stature, and bore a healthy calf sired by Bull #2 above from a Fall 2000 breeding. Bull #2 was also Cow #3's great uncle on the sire's sire's side. Cow #3 could only have been infected upon Ranch A by close animal contact, but the stage of disease, plus the lack of other organ confirmation suggests that additional confirmation work needs to be done. The author has requested a confirmatory DNA tissue comparison, but that has not been done to date. The feed used at Ranch A was predominately ranch-grown, or secondarily purchased locally, together with bulk, locally grown oats and manufactured wheat-middlings mineral pellet. As of February 2003, no wild animal CWD was been found within 150 miles of Ranch A, nor its sources of feed.

Ranch A's calf #1c and the calving crop events surrounding calf #1c are potentially pivotal in the study of CWD, its agent and transmissibility. Calf #1c, a heifer, was born June 3, 2001 to Cow #1 which died of terminal CWD on August 31, 2001. Calf #1c was small and sickly from birth with diminished physical capacity. Strikingly, it possessed a left unilateral light blue cataract from birth and was continually plagued from birth with a clear to light yellow opaque nasal discharge. After 37 days with gradual emaciation, calf #1c

died and was necropsied in great detail. The final determined cause of death was malnutrition. A CWD brain test was negative, as were other requested testing procedures.

Commencing seven days after the birth of Calf #1c (June 10, 2001) a serious pathogenic mechanism appeared Ranch A's calf crop of the Cow #1 birthing pasture. Cow #1 pasture was composed of mostly new mothers (1999 heifers, a few 1998 heifers) and a few other cows, which had missed calving in the prior season. Of the 52 calves born in this birthing pasture, 19 calves were born prior to June 10th (including calf #1c) and of them, 3 had died of unrelated causes (15.8% mortality), one an abandoned bottle calf, one born dead and calf #1c itself. Of those calves born after June 10th (33 calves) fully 20 calves (61%) died generally within three weeks of birth, as well as, one born dead. This compares to Ranch A's year 2000 mortality rate of 5.7% (three late calves out of 53) and a 1999 rate of 11.6% (five head from a total of 43, of which three were birthing problems).

The symptoms on the majority of 2001 calf deaths were in order of appearance: clear to white nasal discharge, followed by erratic yellow to white diarrhea, collapse, coma, no or only mild temperature (100 to 101° C), occasional convulsions and rapid onset of death within 72 hours of the first symptom appearance. Death appeared to be from hypoglycemic shock or septicemia, but no confirming diagnosis could be obtained at necropsy. Random necropsies found erratic Rota-virus and Corona-virus, as well as, anomalous adenovirus within selected animals but with no common malady amongst the group. All the mother cows had been treated with 7-way shots a few weeks prior to calving, and several of the calves, most of which later succumbed, had been given oral Rota-Corona and E-coli vaccines and subcutaneous C&D clostridial injection within 12 hours after birth, well prior to noticeable disease symptoms.

No obvious cause of death could be confirmed in any of the calves. General neo-natal dehydration was present, however, even keen hydration therapy (saline and/or glucose) failed to save or revive comatose calves. Heavy dosage antibiotic treatment, including penicillin (several kinds), gentamycin, trimethaprin, florfenicol and a steroid treatment were used to no avail. Forced feeding of bovine colostrum prior to coma did prolong life for up to 10 days, however death resulted within 24 hours following colostrum withdrawal. A single sick calf, nursed by an independent animal boarder reportedly lived for an extended period of time after reportedly being given tetracycline as LA-200.

Of the 33 calves born in the infected pen after June 10th, 20 died, but the 13 that survived had a few common traits. A few (four or five) had seemingly survived the nasal discharge stage, three (of the only three on the farm) were from one gene pool derived from non-ranch-raised purchased pregnant heifers, and one quite healthy, unaffected calf that was the offspring of cow #3 mated to bull #2 above. An attempt to preserve 11 of these seemingly healthy escapees from the calf pathogen for use as progressive CWD research at a private, secure endemic area facility was rejected by the USDA and all eleven were summarily killed at 9 months of age with no CWD positives.

Ranch C: Larimer County, Colorado

Ranch C is located at the south central boundary of Larimer County, Colorado near the Little Thompson River, a main tributary of the South Platte River. Ranch C had two ranch-born CWD positives, a male and a female both born in June of 1999. The clinically normal (other than deformed antler) male was slaughtered for meat in early September of 2001 at the age of 28 months, while the female died at the ranch of CWD-induced complications in early August 2001 at the age of 27 months. The slaughtered bull's brain sample revealed the early stages of CWD and the hanging carcass was impounded and destroyed by the Colorado Department of Agriculture. The remainder of the herd was destroyed under the USDA CWD indemnity program but revealed no further positives. The bull was of average size and weight prior to slaughter, as was the female prior to the occurrence of CWD clinical signs about 4 months prior to death. The female had given birth to a seemingly healthy calf in June of 2001, which died 3 days after birth due to owner-perceived maternal neglect. Prior to the birth of the positives, alfalfa pellets manufactured at Greeley, Colorado provided herd feed. Subsequently, herd feed was ranch-grown or locally purchased alfalfa hay. Supplemental feed was purchased as a pre-sacked mixture of oats-corn-barley with minerals, reportedly derived from a Canadian source. According to CDOW sampling reports, a wild animal CWD hot spot with multiple infections has recently been identified only one mile west of the Ranch C single fence elk pens.

Ranch D: Larimer County, Colorado

Ranch D had a single, purchased male elk born in June of 1999, and brought to the ranch in February 2000 at the age of 8 months. Ranch D was single-fenced for elk located in southeastern Larimer County in an area regarded as well infected with wild CWD. The original source ranch was located in southeastern Weld County within the recognized Colorado CWD endemic area, but in an area of a lower incidence rate then that at Ranch D. The source ranch had numerous animals from Ranch B, but those animals were depopulated under the Fall 2001 trace back phase of the USDA program with no positives. Under the Spring 2002 voluntary USDA CWD depopulation program, the remainder of the source herd was depopulated with no positive animals. The single positive animal at Ranch D was clinically normal in the spring of 2002 when its velvet failed to develop properly. Scruffy, brittle hair coat, poor antlers and a failure to gain weight on good feed preceded the minimal clinical signs of head drooping and avoidance of the remaining herd which became noticeable a few weeks prior to termination via gun shot in August of 2002 at an age of 36 months. The remainder of the herd was still alive with no obvious clinical signs as of February 2003. A concerted effort is being mounted to retain the Ranch D herd as an insitu CWD research herd. Assuming a postulated CWD incubation period of 18 to 32 months, the positive animal could have acquired its infection right at Ranch D or much less likely at the source ranch, which however had no other positives. The feed supplied from Ranch D came from locally packaged alfalfa pellets, timothy hay from North Park

and/or the Galeton area (central Weld County, just north of S. Platte River) and prepackaged sacked oats of unknown origin.

Several additional cases of spurious domestic CWD were found during the Summer 2002, indemnified voluntary depopulation of several elk ranches in northeast Colorado, all in Weld and Larimer Counties. The presence of these seemingly out-of-the-blue positives occurred upon two separate ranches, three cases in southeastern Larimer County and one case in northeastern Larimer County. Both ranches were well within the area of "endemic" high wild disease rate incidence and were regarded as transferred from the wild. One used all locally grown alfalfa hay, the other used hay derived from the extreme southwestern corner of Nebraska, as well as some additional forage locally grown. The Nebraska location was later found to have wild cases of CWD and is now regarded as part of the endemic area.

Central Alberta, Canada

Moving on to other cases outside of Colorado, in July 2002 a sub-clinical CWD-infected elk slaughter bull was found in central Alberta, Canada. When its several-hundred herd mates were destroyed, no other positives were found, a seemingly isolated case giving credibility to the theory that maybe CWD is just a natural disease in the wild. However, one must look a bit deeper into this case. It has been reported by several individuals that this particular herd had, prior to 1990, sourced animals from South Dakota, in particular, animals from a South Dakota herd that had been later diagnosed with CWD. Although the particular animal from that South Dakota herd was destroyed in 1989 due to a TB depopulation program, that animal had been resident for an extended period of time. Seemingly, the 2002 case may have acquired CWD from a residual environmental source.

South Dakota

In August 2002 the South Dakota Animal Industry Board reported that Chronic Wasting Disease has been detected as part of routine slaughter surveillance on a 3-year-old bull elk from a private elk ranch in southwestern South Dakota. A second herd, immediately adjacent to this herd had been quarantined and eliminated 51 months previously due to CWD. The current CWD animal was from a double-fenced group, which had been quarantined for 4 years as a precaution due to brief fence-line contact with the positive adjacent herd during 1998. Following 52 months of close surveillance, the herd was considered not at risk. The new source of disease was not readily determined, as the elk found positive was born 15 months following the depopulation of the adjacent herd. Surveillance sampling done on 23 animals over the previous 52 months was negative, as was the positive bull's dam when slaughtered in December 2000. The remainder of the herd was in good health providing little evidence as to the source of disease. (SDAIB, Press Release, 8/16/02). Unverified reports suggest that a herd sire derived from the adjacent positive CWD herd may have been resident for a short period of time at the subject ranch prior to the adjacent ranch's depopulation over 51 months previous.

Importantly, several similar, but less well-documented cases have continued to confound and confuse the veterinary community both in wild animals and in captive or domestic cervidae. Other than newspaper reports of erratic quality, detailed information on most cases is not readily available due to the privileged nature of on-going governmental investigations and the need to preserve the privacy of individuals or private business establishments.

DISCUSSION

The cases histories of most TSE diseases and in particular CWD have been at times, conflicting and even contradictory under some circumstances. As such, the epidemiology of CWD remains clouded and is in dire need of more comprehensive study. In the few cases examined herein, a common thread emerges. The etiological agent is unrecognized or unrecognizable and the mode of transmission is unknown. Further, the agent is seemingly persistent in the environment. A close review of the captive and domestic cases can shed light upon the CWD conundrum.

The records of the Colorado Division of Wildlife pens and the nearby CSU pens, both at Ft. Collins, Colorado are the records of the most CWD-contaminated facilities in the world. Despite de-contamination attempts, CWD has been and remains the most prominent adult cervid mortality factor at these government-operated pens. High elk calf and deer fawn mortality preceded and paralleled CWD mortality. Trindle (PC, Jan., 2003) noted that several newborn dietary changes failed to alleviate neo-natal losses, and now believes that neo-natal stress associated with multiple fawn handlers might have lead to excessive newborn mortality, but could not rule out the role of an unknown infective agent.

Ranch A may have randomly presented the scientific community with an incredibly crucial epidemiological event, never previously recorded in TSE research. That event centers on the quite distinctive and highly anomalous presence of a clinical abnormal calf born to a terminal CWD elk cow. That birth was shortly followed thereafter by the subsequent mortality of numerous neo-natal pen mates (post June 10^{th} calves). An important question must be asked. Was this abnormal calf simply a co-incidental event, or was this calf suffering from an in utero infection by the agent of CWD, or perhaps a closely affiliated commensal agent? Was that agent a Spiroplasma?

Intriguingly, the presence of a unilateral cataract in calf #1c, as it was co-temporally affected by rhinitis and enteritis may parallel the findings of Kern (1998) in regards to the neonatal human symptoms derived from a Spiroplasma infection. In that case, Kern documented the only known case of an active Spiroplasma infection of a human, which showed a rapidly progressing unilateral cataract associated with severe inflammation of the iris in a premature baby at 4 months of age. Although cell cultures were negative, PCR DNA sequencing identified a positive marker for Spiroplasma. Prior to birth, the mother suffered repeated vaginal infections presumably caused by a mycoplasma and the baby

sustained a suspicious bout of pneumonia and respiratory distress of unknown etiology at three months of age immediately preceding cataract development.

One must compare the symptoms of calf #1c with the results defined by Tully (1982), where suckling rats were inoculated with *Spiroplasma mirum* SMCA strain and high doses were fatal due to septicemia while lesser dosage showed a high incidence of ocular cataracts. Using the *S. mirum* GT-48 strain Tully also found low-dose pathological septicemia. In 1984, Tully intracerebrally inoculated neonatal rats with GT-48, but only a small percentage survived more than 14 days. Again septicemia was implicated. Bastian (1984) scrutinized the infected brain material of Tully's GT-48 inoculated rats, and noted but little inflammation, some neuronal vacuolization, yet widespread dilation of neuronal processes, but no detectable Spiroplasma organisms. Additionally in 1987, Bastian demonstrated that subcutaneous inoculation of the GT-48 strain produced hair loss and a diminishment in body weight (runting), yet produced both unilateral and bilateral cataracts. Prominently, the relationship of Spiroplasma to cataracts is unequivocal, and the symptoms expressed by Ranch A's calf #1c were certainly conducive to a Spiroplasma etiological agent.

A second factor notably affecting Ranch A was its staggering late season neo-natal mortality. The calf loss rate at Ranch A was excruciatingly high commencing 7 days after the June 10th, 2001 birth date of calf #1c. While calves born prior to June 10th were unaffected, those born after were highly at risk. Prior to 2001, Ranch A's calf mortality rarely exceeded 10%, generally much less, most deaths attributed to birthing stress. While no specific agent could be identified in 2001, the general calf mortality pattern was conducive to the occurrence of a Spiroplasma septicemia epidemic event mimicking Tully's (1984) severe neonatal rat mortality. The uncanny presence of cataracts on a neo-natal calf born to a virulent, end stage, CWD-infected mother tantalizes at the possible role of *Spiroplasma mirum* (SMCA or GT-48 strain) or a similarly structured close relative as a potential roll player in the CWD drama that unfolded at Ranch A.

While the obvious temptation may be to ascribe the generalized high calf or fawn mortality to continued poor cervid management or an opportunistic, resident neo-natal infectious agent, such as E-coli or Rota-Corona virus. Ranch A calves were vaccinated against such pathogens. The high mortality rates experienced the Ft. Collins facilities, over several years, betrays what seems to be a serious underlying contamination. Coincidentally, such contamination is ostensibly the same as, or is fully co-existent with the ever present and serious CWD infectivity. Between 1968 and 1976, a cumulative +60% mule deer FAWN mortality (broadly due to unknown etiology) was followed by a 79% ADULT mule deer CWD infection rate as documented between 1974 and 1979. The majority of CWD-related deaths occurred by three years of age. The only other major cause of death among the remaining 21% of adults was physical injury. The neonatal mortality agent and the CWD infectivity were co-existent.

Salient Characteristics of Selected Wild and Domestic CWD Occurrences

Positive CWD Facility & Location	Detection Time Frame	Potential Residual Agent	Infective Import Animal	Endemic Area Feed	Potential Water Agent	Potential Insect Agent	Severe Neonatal Mortality
Ft. Collins DOW & CSU Pens, NE CO	1968 2002	Yes	Yes	Yes	No	Yes	Yes
Denver Wildlife Resch Ctr, NE CO*	1961? 1974?	Unknown	Yes	Yes	No	Yes	Yes
Ranch A, SW CO	2001	No	Yes	No	No	No	Yes
Ranch B, NE CO	1998-2001	Possible	Possible	Yes	Possible	Yes	No
Ranch C, NE CO	2001	Unlikely	No	Yes	Possible	Yes	No
Ranch D, NE CO	2002	Unlikely	No	Yes	No	Yes	No
Cent. Alberta Ranch	2002	Yes	No	No	No	Yes	No
South Dakota Ranch	2002	Yes	No	No	Possible	Yes	No
New Mexico Wild	2002	Yes	Unknown	Yes	No	Yes	Unknown
Western CO Wild	2002	Yes	Yes	No	No	Yes	Unknown

** Prior to CWD recognition, not diagnosed, but suspected by Author*

As reported with somewhat incomplete data, between 1968 and 1979 of some 258 documented mule deer born or housed at the DOW Ft. Collins pens, at least 147 died as young neo-natals (57.0%), 53 died of CWD (20.5%), 14 (5.4%) died of other causes (usually by accident). The remainder, some 44 animals (17.1%) were unaccounted for, apparently dying of unknown causes, moved to other facilities, or were returned to the wild. Hence, an incredible 200 of 214 (93.5%) well-documented mule deer died either as neo-natals prior to weaning (68.7%), or died as CWD-infected adults generally prior to 3 years of age (24.8%). More recently, as of mid-1998, Miller (7/98 Wildlife Research Job Progress Rept.) noted that CWD accounted for 83% of adult mule deer mortality, and 100% of the whitetail deer mortality at the FWRF.

Such a horrendous mortality rate is particularly curious and unlikely to be purely coincidental, particularly when compared to the extraordinarily similar circumstances experienced at Ranch A in 2001. This pattern parallels the superficially analogous circumstances at the U.S. Fish and Wildlife's Denver Wildlife Research Center in the late 1960's. While the evidence is purely circumstantial, it is without definitive identification of a specific etiological agent. As such, the evidence is permissive for a distinct relationship between neo-natal mortality and adult CWD mortality, particularly in closely confined facilities. The short duration of clinical disease, commonly leading to CWD-related deer deaths at 18 to 25 months of age suggests infections as neo-natals. An

infection that is basically contemporaneous with the undetermined etiologic neo-natal mortality.

A close review of CWD cases at example Ranches C and D is indicative of the observed out-of-the-blue nature of CWD occurrences in Colorado. Numerous additional instances have been documented, but to a lesser-detailed extent. Collectively, CWD has appeared upon ranches in cervid herds without recognizable trace-backs to known or potentially infected herds. When depopulation is undertaken, no cohorts are infected and the disease incident is seemingly random in nature, almost appearing to be spontaneous in nature. But is it spontaneous or is there a common thread?

Certainly the wild cases and most of the domestic elk cases, particularly those in the Colorado endemic area can potentially be explained by simple nose-to-nose contact. However, the chance of contact between infected mule deer and elk is quite unlikely due to the dominant nature of elk when in the presence of deer. The deer quickly move away from elk when discovered. While nose-to-nose contact is possible between wild elk and domestic elk, the seemingly transitory nature of exposure would be in contravention of the perceived intensive exposure necessary to infect older animals as determined by Miller (1998). Transitory contact, however, could be a factor for neo-natal elk, which lack an immune system and quick exposure may be sufficient to pass the agent.

A second factor might be the presence of residual soil or vegetation infectivity, either shed by an infected animal during life, i.e. feces, urine or saliva, or upon death from the decay of carcass matter. We know that soil-resident infectivity may be possible. Research has shown remarkable agent refractivity to natural processes. Pattison and Millson (1961) documented survivability in numerous freeze-thaw cycles. Brown and Gajdusek (1991) have demonstrated that scrapie infectivity homogenate can survive for over 3 years in buried soil conditions.

The infectivity of a TSE-infected carcass is assured. Certainly, the nature of experimental TSE infection directly uses carcass material, brain, blood, spleen, etc. to inject or force ingestion of TSE agent with considerable positive results. However, to date, few direct infectivity studies have been done upon potential TSE shed material, such as urine, feces or saliva, let alone sheds particular for CWD. None have yet shown to be successful in transmitting TSE (Gabizon, 2001). Interestingly, although experimental alimentary TSE infection is well documented, no causal agent has been specifically identified, that is other than, the ubiquitous, but dubious infective prionic protein. However, specific studies to find abnormal prions in blood, urine and feces have been unsuccessful, yet the fact remains that blood, placenta and colostrum are TSE infectious (Tamai 1992). Obviously, this suggests that a prionic agent is either just not present or is virtually undetectable at the concentrations available in the subject tissue. As such, it would seem that the postulated prionic agent, if present at all, is of insufficient infectivity in sheds to produce disease, or possibly that the risk of contact and potential acquisition of the pathogenic agent via sheds is minimal.

Now, since carcass infectivity is assured, can it be a factor in transmission? While the temptation would be assume the affirmative, and certainly one cannot rule out carcass transmission, its role in sustaining the TSE contagion can only be minimal at best. While some have suggested that cervids gnawing on old bones and or antler for needed calcium may play a role in wild passage, it certainly cannot explain domestic passage. The remainder of a carcass could indeed create passage if ingested, but surely the dietary habit of cervidea generally excludes cannibalized animal matter. While the agent of BSE is likely passed via rendered animal protein feed products, the sustaining BSE epidemic in Great Britain, albeit at much lesser rates, well after a ruminant feed ban suggests that other possible, but less obvious routes of transmission may be at play. Lastly, the initially random and then sustaining nature of domestic cases cancels many of the suggested disease diffusion routes. In farmed cervids, prohibited ruminant feed products have generally not been used, intense management precludes cervid cannibalism and the quite unlikely nature of sporadic residual wild carcass infectivity being selectively surrounded by randomly fenced domestic pens is of predictably low probability.

So where is the commonality? Perhaps air, water or feed may play a role. While air transmission is documented for many pathogens, such is not the case for TSE. Close, rather intense animal contact or near contact is necessary, basically precluding distant airborne travel. If nothing else, aeolian transport sustains incredible particulate dilution ostensibly rendering the agent insufficient for infectivity. Some domestic cases particularly those of Ranches B and D suggest that a water or water-borne sediment transport might be possible, however, like aolean transport, sediment transport sustains severe particulate dilution, again likely to render the agent ineffective. Straight, water borne passage has suggestions of favor, but the nature of infection upon ranches with all animals using common well water sources, yet discrete outbreaks of disease, hints otherwise as well portrayed at Ranch B. Further, the CDOW FWRC and the CSU WDRC facilities both used domestic city water, as did the USFW's DWRC. Stagnant water agent availability or perhaps agent replication appears plausible, but only on Ranch B and at a northwestern Nebraska fenced ranch where wild caught white-tailed deer with only one spring-fed water source, had an extraordinarily high infection rate.

Feed has thus become the wild card. Obviously, all wild and domestic ruminants ingest feed. Some from common sources, some from very divergent sources. The documented infected domestic ranches use several feed components in day-to-day activities, listed in order of abundance; 1) forage or hay, 2) grain supplement and perhaps, 3) a mineral pellet or top-dressing. None knowingly used banned ruminant-derived supplements, nor is there commonality in use or source of mineral supplement, although any cervid formulated mineral supplement likely contained added copper. Further, no common lineage can be found in grain allotments. Some feed oats, some feed corn, some grain was grown locally, some imported from Canada, and still others were derived from mid-western states. While feed grains themselves could conceivably contain a pathogenic bacteria, perhaps incorporated as a close cousin of Spiroplasma-caused corn stunt disease, at this point the

link would be very tenuous. Grains can also contain an abundance of astigmatic mites, which when exposed to the TSE agent are known to be carriers of TSE infectivity (Forrest, 2003a). While a common grain link has yet to be ascertained, the role of grains and mites needs additional investigation. This now leaves us with forage or hay.

Due to financial constraints, virtually all domestic cervid breeding ranches confine cervids to pens of relatively small aerial extent, from one to 200 acres and high animal stocking rates (0.5 to over 5 per acre). Natural forage is generally unavailable, most or at least part of the year, hence, all will supplementally feed animals with commercially produced hay, usually either grass or alfalfa. All Colorado endemic area ranches used hay and/or grazed animals upon land located in the South Platte River drainage. The South Platte drainage is fully within or coincident with the initial Colorado-Wyoming wild CWD endemic area.

Commercially grown hay in the South Platte basin, with its irrigation and fertilization is prime grazing ground for wild cervid species. Hot spots of wild disease may occasionally overlie areas of intense irrigation. Undoubtedly the excrement, residual products and even carcasses of wild-infected CWD cervidea pollute the soil of South Platte grasslands and on-going hay operations.

As presented in Part IIa "Novel Vectors"(Forrest 2003a) grazing pastures contain mites and numerous other insects. TSE contaminated pastures contained infected mites, some of which harbor the infective causal TSE agent. While only minimal arthropods studies have been conducted in Colorado, certainly commercially produced hay, and the fields from which it is produced, contain many opportunistic insect species, in particular forage, grain, mold and grass mites. If mites are capable of acquiring by contact the causal TSE agent from the residual products of diseased wild or domestic animals, then a potential vector for the transmission of CWD has been established.

In the domestic animals of Ranch B the initial cases and the sporadic reoccurrence thereafter, is potentially conducive to a feed-introduced and thence, field-propagated agent. But at Ranch B the possibility of introduced disease via numerous imported animals from the Colorado endemic Front Range is also possible. Certainly, Ranches C and D (as well as several others undocumented cases) are better evidence of a surreptitiously introduced agent, possibly by the feed. No correlation with imported animals can be documented.

The Central Alberta case finds that a prior potentially-infected animal may have shed the causal agent into the pasture environment. Perhaps such agent remained either in a conserved form or as self-replicating, sustaining agent, which was then reintroduced into a susceptible cervid many years later. South Dakota is a similar case, where adjacent pastures contained infected animals later depopulated, yet several years later the disease reappeared in an adjacent pasture. Simply put, the agent, perhaps resident within it a possible insect vector could have easily traveled the short distance between pens to infect a young animal unexposed to the prior positives.

The on-going sequence of Colorado's captive wild animals at Ft Collins perhaps mimics the South Dakota example. While residual ground contamination can not be ruled out, and despite disinfection procedures potentially eliminating the disease and its vectors, the agent was possibly reintroduced into the pens by a mobile insect vector. Such a vector derived from adjacent fields, essentially following the slow and inevitable re-introduction of ground cover vegetation within the plowed and disinfected pens. More logically, the Ft Collins pens obtained locally produced forage feed derived from potentially infected South Platte River hay fields. A similar situation may be present at the Wyoming research facility. The five-year lapse in CWD infectivity in the government pens was not caused by a long disease incubation period, but simply represents the time necessary to re-introduce the agent via flying or walking insects, or more likely, via hitching a ride within contaminated baled forage.

The western Colorado CWD introduction was then possibility due to: 1) residual infectivity present from limited east-west animal migration over the continental Divide, and more likely, 2) the CDOW-provided transportation of infected captive cervids to western Colorado locations with the subsequent shedding of infectivity into the environment. Basically, CWD-infected urine or feces providing a source of CWD infectivity later assimilated by a resilient, resident insect vector. Grazing studies using CWD infected animals over a portion of Middle Park and in the Little Snake River area became loci of infectivity, with the vector-ensconced causal agent lying in wait. The vector then sustained the CWD infectivity in the environment via reproduction within the insect corpus, passed down via generation after generation until eventually, an unfortunate new cervid host materialized. That led to direct CWD infection and the diagnostic Transmissible Spongiform Encephalopathy.

The recent (February, 2003) southern New Mexico CWD outbreak (6 out of 26 samples or 23%) is tantalizingly unique. The initial case was diagnosed in the central portion of the White Sands Missile Range, but later cases appeared to the south and west across the San Andreas Wildlife refuge and into the western margins of the Organ Mountains, east of Las Cruces, New Mexico. Interestingly enough, the I-25 corridor between Las Cruces and El Paso Texas, paralleling the Rio Grande River, is the home to tens of thousands of dairy cows, virtually the milk bucket of the American Southwest. A major source of the high quality feed necessary to produce this abundance of milk, are the rich, high altitude alfalfa fields of Colorado. Daily, hundreds of tons of alfalfa hay are shipped south, predominately from the San Luis Valley of southern Colorado, but also from sources extending north all the way along the I-25 corridor, well into the northeast Colorado Front Range endemic area. Northeast Colorado is seemingly the initial home and likely source of all continental Chronic Wasting Disease. Could transported Colorado hay containing residual infected insects be the source of New Mexico CWD? Additional investigation is justified.

The collective data elucidated in Parts I, IIa and IIb (Forrest, 2002, 2003a, 2003b) will be forged into cohesive hypothesis of CWD transmission to be presented in **Chronic Wasting Disease -** Part IIc "**Transmissible Postulates"**.

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