*15th November 2003.*

*To; THE FATS AND PROTEIN RESEARCH FOUNDATION.*

*THE FINAL REPORT OF AN ANALYTICAL STUDY INVESTIGATING THE LEVELS OF 46 METALS IN THE ECOSYSTEMS SUPPORTING CLUSTERS OF TSE IN NORTH AMERICA 2002/2003;*

**( Additional reports/articles on the comparative magnetic / radioactive status of the soils / antlers drawn from TSE/TSE-free areas of N America during this study will follow over the next weeks)**

*The following report has been submitted as an article to the Vetinerary Record – the journal of the British Veterinary Association;*

*Short communications;*

# Elevated silver, barium and strontium in antlers sourced from CWD affected herds.

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Exceptionally high levels of silver (Ag), strontium (Sr) and barium (Ba) were measured in deer antlers, vegetation, soils sourced from chronic wasting disease (CWD) cluster areas in Colorado, Wisconsin, Saskatchewan , whilst moderately high to mean levels were measured in CWD-free areas of Alberta. Ag could not be detected in antlers collected from the CWD-free British isles. These observations were recorded as part of an extensive comparative analytical study of the levels of 46 metals in the soils, water and vegetation of CWD cluster and CWD-free regions across North America. This work represented the N American perspective of a three year globally orientated project designed to establish whether any abnormal mineral profile or abnormal magnetic / radioactive /oxidative capacity is a common characteristic of TSE cluster ecosystems around the world, and, if so, whether that abnormality plays a primary role in the pathogenesis of TSEs .

Since the origins of TSE are unknown, this study was designed to challenge the theory (Purdey 2002, Purdey 2003) that high levels of specific metals, such as manganese or Ag, in combination with low levels of copper (Cu) in the environment may bring about a rogue metal replacement at vacant Cu ligands on the cellular prion protein (PrPc) – a Cu binding protein ( Brown 2001) whose misfolded isoforms hallmark the TSE diseased brain ( Prion diseases 1997). Since PrPc has been shown to perform some metabolic role in mediating the circadian rhythm (Tobler and others 1996), this theory proposed that an aberrant metal replacement of Cu on PrP's metallo domains disrupts the putative role of PrP's Cu component as a ***conductor*** of electromagnetic radiation ( transduced from incoming light and sound ) around the circadian mediated circuits of the biosystem ( Purdey 2002, Purdey 2003) which delivers the 'spark' to the sulfated proteoglycan (heparan)-fibroblast growth factor signalling system; the all important system which maintains the growth and structural integrity of the nervous system ( Kan and others 1996).

In this respect, the pathogenesis of TSE could be initiated by a disruption at any point along these 'conduits' of electromagnetic conduction; such as a replacement binding by a foreign metal on PrP's native metal domains, where substitution by a rogue ferrimagnetic ( or diamagnetic ) metal species disrupts the normal paramagnetic conducting capacity of PrP's Cu co-partner. The newly acquired ferrimagnetic ordering at PrP's metal domains initiates a permanent force field of magnetic induction, which progressively corrupts the circadian / acoustic / vestibular circuits, inducing a contagious domino-like aggregation of metallo-PrP molecules into crystalline fibril structures, with a consequent collapse in the sulphated proteoglycan / FGF mediated signalling and antioxidant systems (Kan and others 1996). TSE pathogenesis ensues.

Antlers from 2 to 3 year old free ranging / farmed cervidae were collected during April-June 2003 across the regions/farms where the most intensive outbreaks of CWD had been officially identified (DNR 2003). Samples were batched according to CWD cluster region and sent to the University of London at the Royal Holloway, Egham, Surrey, UK for chemical analysis. The samples for analysis were ignited to 600°C to remove organic material and then powdered in an agate mortar and pestle. They were then dissolved in hydrofluoric and nitric acids and analysed by Inductively Coupled Plasma Atomic Emission and Inductively Coupled Plasma Mass Spectrometry.

High levels of Ag and low levels of Cu were recorded in the antler material, soils and deer browse vegetation drawn from CWD affected zones (see table 1and 2). These results represent the first time that Ag has been detected in antlers, whilst adding some support to the proposal (Purdey 2002, Purdey 2003) that high Ag and low Cu in the environment may bring about an Ag replacement of vacant Cu ligands on the cellular prion protein (PrPc).

Ag is potentially highly toxic (Petering 1976), exerting a strong competitive binding affinity for specific Cu ligands on cuproproteins ( Ghandour and others 1988). The degree of intoxication encountered following Ag exposure is controlled by the overall Ag/Cu ratio within the biosystem.

This novel observation of elevated Ag in antler suggests that the antler acts as a depot for the bioaccumulation of Ag or other heavy metals in deer who are thriving upon metal contaminated foodchains. The toxic load is conveniently shed along with the antler on an annual basis.

Apart from the naturally occurring sources of Ag in soils, possible routes of Ag exposure in the CWD cluster ecosystems could stem from routine feeding of Ag contaminated concentrated feed pellets to captive and wild deer herds. In this respect, Ag was measured at 2.2 ppm in the feed samples collected from deer farms across North America during this study (see table 1). Another significant source of Ag contamination in the drought prone regions where CWD has emerged stems from the extensive aerial spray application of silver iodide crystals used as founder nuclei in cloud seeding ‘rainmaking / snowmaking’ operations (Cooper and Jolly

1977). The resulting Ag contaminated rain permeates the local vegetation as well as the growing crops that are incorporated into the deer feed.

Whilst airborne Ag can be absorbed directly into the brain via the nasal-olfactory route of inhalation (Takenaka S and others 2001), Ag bioaccumulates in Bryophytes ( lichens/mosses concentrate Ag up to 9ppm (Cooper and Jolly 1970)) and other vegetation ( see table 2), which are subsequently ingested by the local deer / elk populations.

It is interesting that the practise of cloud seeding is largely contained within the North American continent – the area which has hosted virtually all cases of TSE in wild animals – whereas use of silver ions as a biocide (Gupta and Silver 1998) has been viewed with greater caution by the US authorities resulting in the greater use of Ag as a biocide within Europe; where it has been used as a water purifier and sterilising agent in the London zoo, Rendering plants, hospitals, etc – Establishments which have been associated with high incidences of TSEs.

The other unusual observation resulting from this study implicates the elevation of Ba / Sr and low levels of sulphur in the antlers, vegetation and soils of the CWD affected deer. Whilst an insufficient number of studies have been conducted on the levels of these metals in antlers (Bubenik 1971, Hyvarinen and others 1977) in order to establish mean reference ranges with any accuracy, mean ref levels of Ba / Sr in bone have been used instead . In respect of the mean levels of 5ppm Ba / 52ppm Sr in bone matrix (WHO 1990, mineral tolerance 1980, Pais and Jones 1997), the 140 ppm Ba / 110 ppm Sr mean levels recorded in the CWD antlers in this study could be regarded as 'elevated'.

Raised levels of Sr have been previously recorded in a study on antlers (Gelbke 1972) where exposures to atmospheric contamination by radioactive Sr 90 was considered to be responsible. If the high Sr recorded in the antlers in this study turns out to stem from a radioactive Sr 90 source, rather than the stable Sr88 form, then the contamination of the Northern Hemisphere by Sr 90 due to the 1986 Chernobyl accident and the 1960s/70s nuclear weapons testing (Eisenbud and Gesell 1977, Whicker and others 1966) combined with radioactive leaks that are specifically local to the CWD cluster regions (Hiatt 1977, Arthur and Alldredge 1979) – may all be contributory to the high levels of Sr recorded in the antler material in this study.

The mean levels of Ba and Sr were significantly higher in the vegetation of the CWD cluster environments at 44 ppm Ba and 62.2 ppm Sr in relation to levels of 27.5 Ba and 16.3 Sr recorded in CWD-free control areas. These were several fold higher than their mean reference levels of 10 ppm Ba and 20 ppm Sr (Underwood 1977) for vegetation. A high Ba / Sr and low sulphur mineral profile has also been recorded by the author in TSE cluster ecosystems in Southern Italy, Japan and Iceland.

The elevated Ba levels in the North American CWD clusters derives from the dolomite / limestone and Cambrian granitic mica schist soil types of the CWD cluster areas. These light, low organic matter soil types are naturally high in Ba and Sr (WHO 1990), whilst being notoriously low in sulphur and copper. The low sulphur perspective exacerbates the problem of Ba / Sr toxicity in the mammal who is dependent upon these foodchains, in that an available source of free sulphur in the soil will conjugate with Ba and Sr, thereby locking up those minerals and acting as a 'toxic sink'/ preventative against Ba intoxication (Pais and Jones 1997).

The customary spreading of spent barium drilling mud across farmland ( a waste product of the fast expanding oil and gas well industry in the CWD areas ) has compounded the problem; with subsequent uptake of Ba into the pasture and hay crops which are ingested by local cervidae populations. Cultivated plants such alfalfa / soy bean, as well as the wild 'locoweed' flora are prevalent in the CWD areas. These species are renowned to bioconcentrate Ba and Sr to high levels (WHO 1990, Shacklette 1978, Mineral tolerance 1980).

It is interesting that conditions of prolonged drought have preceded the outbreaks of CWD in N America. This could stem from the fact that drought conditions exacerbate the problem of metal bioconcentration in grazing deer, in that the resulting shortages of pasture cause malnourished cervidae populations to consume abnormally high intakes of pine, juniper and locoweed as a substitute for their normal rations – a phenomena that is widely reported by hunters and ranchers operating in the CWD environments. These plants bioconcentrate Ba and Sr ( WHO 1990 ). Furthermore, once the deer are forced to compete for the dwindling reserves of close cropped, drought-parched pasture, their intake of topsoil - and the metals contained therein - is dramatically increased (Arthur and Alldredge 1979, Mayland and others 1975). Interestingly, increased amounts of soil and grit have been observed in the digestive tracts of CWD positive deer in relation to deer who have died of other causes ( Williams and Young 1992 ).

In this respect, it has been suggested that an elevated intake of Ba and /or Sr may play some primary role in the multifactorial aetiology of TSEs (Purdey 2003). The soluble salts of Ba and Sr are highly reactive and will readily conjugate with sulphur in the biosystem (WHO 1990); thereby depriving the endogenous sulphated(S)-proteoglycan molecules of their crucial sulphur component. S-proteoglycans have been shown to perform a crucial co-operative role in the fibroblast growth factor mediated signalling system that maintains the overall growth and structural integrity of the architecture of the nervous system (Kan and others 1996). Disrupted S-proteoglycan signalling systems are a consistent feature in the pathogenesis of many neurodegenerative diseases (Kan and others 1996), whilst the demonstration of a metabolic association between the S-proteoglycan molecules and the cellular prion protein (Caughey and Raymond 1993) suggests that the disruption of S-proteoglycan mediated signalling systems - observed in the pathogenesis of TSEs - performs a pivotal role in the origins of TSE diseases. In this respect, it is suggested that this disruption of S-proteoglycan activity is caused by chronic exposure to sub toxic levels of these sulphur capturing metals, Ba or Sr.

Whilst the elevated levels of Ag, Ba and Sr in antlers may turn out to bear no relationship to the pathogenesis of TSEs, the presence of Ag may indicate that the antler acts as a hitherto unrecognised toxic 'sink' for storing excess intakes of Ag in cervidae, or, alternatively, that Ag performs some metabolic role as an electrical ***superconductor*** for mediating the rapid growth of the antler. Likewise, the elevation of Ba and Sr in antlers may indicate a previously unrecognised case of chronic exposure to sub-acute toxic levels of Ba / Sr in the external environment.

More extensive and detailed analytical studies need to be performed in order to reach a more conclusive consensus on these preliminary observations within this interesting area of TSE research.

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| *Matrix* | *sampling zone* | *CWD status* | *Ca0 %* | *Ba ppm* | *Cu ppm* | *Sr ppm* | *Ag ppm* | *Mn ppm* | |
|---|---|---|---|---|---|---|---|---|---|
| Antler | Fort Collins,Co | CWD+ | 25.25 | 138 | 2 | 130 | 3.9 | 0 | (w)(t) |
| Antler | Fort Collins,Co | CWD+ | 25.56 | 125 | 2 | 137 | 3.4 | 0 | (w)(b) |
| Antler | Mt Horeb,Wi | CWD+ | 25.78 | 63 | 3 | 42 | 2.1 | 0 | (w)(t) |
| Antler | Mt Horeb,Wi | CWD+ | 25.43 | 60 | 3 | 42 | 3.0 | 0 | (w)(b) |
| Antler | Manitou, Sk | CWD+ | 25.59 | 206 | 2 | 117 | 4.7 | 0 | (w) |
| Antler | Manitou, Sk | CWD+ | 25.64 | 202 | 2 | 130 | 2.7 | 0 | (w) |
| Antler | Manitou, Sk | CWD+ | 25.29 | 280 | 2 | 114 | 9.6 | 0 | (w) |
| Antler | Lloydminster,Sk | CWD+ | 24.98 | 88 | 76 | 136 | 3.1 | 0 | (F)elk |
| Antler | Lloydminster,Sk | CWD+ | 25.20 | 77 | 3 | 120 | 2.7 | 0 | (F)elk |
| Antler | Lloydminster,Sk | CWD+ | 25.35 | 156 | 2 | 130 | 4.6 | 0 | (F)elk |
| ***Mean CWD antler;*** | | | ***25.46*** | ***140*** | ***9.7*** | ***110*** | ***4.0*** | ***0*** | |
| Antler | Alberta | CWD-free | 25.98 | 56 | 2 | 77 | 2.4 | 0 | (F)(t) |
| Antler | Alberta | CWD-free | 25.35 | 52 | 2 | 71 | 4.2 | 0 | (F)(b) |
| Antler | Alberta | CWD-free | 25.10 | 72 | 2 | 38 | 2.0 | 0 | (w)(t) |
| Antler | Alberta | CWD-free | 24.73 | 69 | 3 | 36 | 3.4 | 0 | (w)(b) |
| Antler | Devon(UK) | CWD-free | | | | | | | (w) |
| ***Mean CWD-free antler;*** | | | ***25.29*** | ***62*** | ***2.25*** | ***55*** | ***3.0*** | ***0*** | |
| ***Reference Mammalian bone;*** | | | ***25*** | ***5*** | ***13*** | ***52*** | ***0.01*** | ***0.2*** | |
| Concentrated deer feed pellets; | | | 1.83 | 16 | 55 | 25 | 2.2 | 222. | |

## Table 1; Levels of metals in antler from CWD cluster and CWD-free zones across North America.

(w) = antler from wild deer herd (F) = antler from farmed deer herd.
(t) = section from tip of antler (b)= section from base of antler.

| *Matrix* | *sampling zone* | *CWD status* | *Ca0 %* | *Ba ppm* | *Cu ppm* | *Sr ppm* | *Ag ppm* | *Mn ppm* | *S %* | |
|---|---|---|---|---|---|---|---|---|---|---|
| Soil | Colorado | CWD+ | 2.65 | 568 | 18 | 192 | .35 | 619 | .27 | (40) |
| Soil | Wisconsin | CWD+ | 1.28 | 477 | 16 | 114 | .21 | 915 | .13 | (40) |
| Soil | Saskatchewan | CWD+ | 1.14 | 905 | 24 | 193 | .27 | 853 | NR | (8) |
| Soil | Vermont | CWD- | 1.99 | 474 | 22 | 98 | .08 | 757 | NR | (30) |
| Soil | Alberta | CWD- | 3.21 | 537 | 17 | 124 | .30 | 550 | NR | (17) |
| ***Soil mean reference;*** | | | ***1.00*** | ***250*** | ***30*** | ***80*** | ***.07*** | ***750*** | ***.30*** | |
| Veg | Colorado | CWD+ | 10192 | 56 | 13 | 61.4 | .459 | 196 | .19 | (40) |
| Veg | Wisconsin | CWD+ | 10288 | 56 | 16 | 57.0 | .858 | 122 | .30 | (40) |
| Veg | Saskatchewan | CWD+ | 11295 | 50 | 4 | 68.1 | NR | 60 | NR | (6) |
| Veg | Vermont | CWD - | 7400 | 24 | 25 | 16.2 | .242 | 111 | .47 | (30) |
| Veg | Alberta | CWD - | 6271 | 31 | 5 | 16.4 | NR | 102 | NR | (2) |
| ***Pasture mean reference*** | | | ***5000*** | ***10*** | ***20*** | ***20*** | ***.05*** | ***50*** | ***.35*** | |

**Table 2; Levels of metals in soils and vegetation sampled across CWD cluster and CWD-free zones.**
(Analyses was performed by MS. Measurements relate to total levels of element recorded as ppm on dry basis. )

(20) = number of sample sites (covering approx 10 acres for each site) involved in the constitution of each mean level of metal displayed above.
NR = not recorded.