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

PART IIa: Novel Vectors

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Abstract: (Part IIa) Transmissible Spongiform Encephalopathies (TSE) and in particular, Chronic Wasting Disease are devastating neuropathologic diseases of mammals, displaying long incubation periods and caused by a unique, but unknown infective agent possessing a high degree of refractivity to normal disinfectant and sterilization procedures. Two insect studies using scrapie infectivity have demonstrated the potential role of insects in TSE disease transmission. Mites and carrion flies are capable of transmitting TSE disease when insect homogenates are injected into, or ingested by lesser mammals. Noteworthy prophylactic laboratory procedures and field collection safeguards have ruled out test subject contamination and yielded TSE agent strains unrecognized to date. Temporal insect collection constraints, together with the variety of insects collected and basic, intimate insect biological processes virtually excludes residual TSE prionic material from being passed down through successive insect generations. Yet TSE infectivity remains, most likely through agent reproduction, resident and independent of the insect host, or its initial exposure. Spiroplasma, a bacterium of the Class Mollicutes is apparently ubiquitous in the insect world. Spiroplasma when injected into lesser vertebrates produces symptoms compatible with TSE disease. The distinct similarity between TSE and insect-derived Spiroplasma neuronal infection, plus the independent and reproducing nature of the TSE agent within the documented insects, demands that additional TSE-oriented Spiroplasma insect-vector investigations and concordant vertebrate impact research must be conducted.

INTRODUCTION

Chronic Wasting Disease (CWD) is a Transmissible Spongiform Encephalopathy (TSE) affecting both wild and domestic cervidea, including elk, mule deer, black-tailed deer and white-tailed deer and whitetail hybrids. All TSE diseases are grouped under the term of "Prion" diseases in recognition of the disease's destructive affect upon a protective protein sheath shielding nervous tissue and the methodology used to detect it. A proteinaceous infective particle (abnormal "Prion" or PrP^{res}) has been postulated as the suspected disease pathogen (Prusiner, 1982), however, other more conventional agents have been offered (Forrest, 2002, Part I).

TSEs are distinguished by: unusually long incubation periods (from months to years); progressive central nervous system degeneration with characteristic histopathological lesions; the lack of an immune or inflammatory response; and unconventional biological and physical properties of the envisaged etiologic agent. Gene sequence analysis of the PrP gene of Rocky Mountain elk suggests that genetics in elk and deer may play a role in disease susceptibility similar to sheep (O'Rourke, 1999).

Considerable controversy concerns the nature of the TSE causative agent and its transmissibility. CWD was first recognized in 1967 as a clinical weight loss syndrome in wild mule deer held captive in government-operated research facilities near Ft. Collins, Colorado. In 1981, distinctive brain lesions were described and associated with "chronic wasting" and were recognized as the pathological signs of a TSE. The clinical signature of CWD includes several non-diagnostic symptoms, such as: weight loss, behavioral changes, excessive water consumption, salivation and urinating, together with erratic teeth grinding (Williams and Young, 1980, 1982, 1992a & 1992b). Some TSEs are thought to be spontaneously induced, others under genetic control, and still others readily transmissible. All can be transmitted through direct intracerebral injection.

In Part I "**A Logical Causative Agent**" (Forrest, 2002) evidence was presented which demonstrated that the scientific community has focused its efforts on understanding and dissecting the abnormal PrP^{res} prion, a symptom of TSE disease rather than the cloaked agent behind it. Additional evidence showed that of all the potential agents described in the scientific literature, 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 prokaryotes lacking cell walls, possessed with an affinity for mammalian host cell membranes, a specific appetite for sterol and phospholipids, a capability of producing highly oxidant hydrogen peroxide, and will biologically metabolize the host's immune system building blocks, all the while, possessing a pleiomorphic character and the capability of slipping into an intracellular "stealth" mode in their bleb phase of morphogenesis. Spiroplasma further have a propensity for the collection of viral infectants, and are possibly capable of being rendered into a bacteriaphage, a bacterial-viral symbiotic relationship, which may have an underlying role in both host invasion and its long-term pathogenesis. Such a relationship may be similar to the role of *Mycoplasma fermentans* and the Human Immune Deficiency virus (HIV) in creating full blown Acquired Immune Deficiency Syndrome (AIDS).

Under the very limited studies conducted to date, diverse varieties of Spiroplasma have demonstrated plant pathogenic characteristics while others are capable of producing neuropathic conditions in vertebrates. Many plant pathogenic Spiroplasmas, i.e. citrus blight and corn stunt rely upon insect passage and multiplication within the insect gut epithelium and salivary glands, while still others are directly pathogenic to the insects, some affecting the sex ratios of the hosts themselves (Tully, 1982). Inoculation of vertebrates and mammals with specific insect-derived Spiroplasma species has revealed a

vertebrate neuro-pathogenicity, particularly in those derived from ticks. A single known case of a natural Spiroplasma infection in a neo-natal human had devastating affects upon various autonomic organs (Kern, 1998).

SPIROPLASMA TRANSMISSION VECTORS

The acceptance of Spiroplasma as a possible causal agent for all TSE diseases discloses an incredibly diverse selection of venues capable of disease agent sequesterance, while providing routes for subsequent disease manifestation. Significantly, an exquisitely diverse multitude of possible vectors are available to this unusual and ubiquitous prokaryote and its immediate invasive cousins.

Plant pathogenic mycoplasmas were initially discovered by electron microscopy in 1967. They are Eubacteria of the class Mollicutes, a group of organisms phylogenetically related to gram-positive bacteria. Their characteristic features reside in the small size of their genomes, the low guanine (G) plus cytosine (C) content of their genomic DNA and the lack of a cell wall. Plant pathogenic mycoplasmas are responsible for several hundred infestation diseases and belong to two groups: the phytoplasmas and the Spiroplasmas (Garnier, 2001). Only three plant-pathogenic Spiroplasmas are known today.

The phytoplasmas (previously called MLOs, or Mycoplasma-Like-Organisms) represent the largest group of plant pathogenic Mollicutes. They are pleiomorphic, and have, so far, resisted in vitro laboratory cultivation making study difficult. Plant pathogenic mycoplasmas are generally restricted to the plant phloem sieve tubes which circulate the host plant's photosynthetically enriched sap, the preferred food source for many phloem sap-feeding insects (aphids, leafhoppers, psyllids, etc.). Interestingly, phytopathogenic mycoplasmas (i.e. Corn Stunt, Citrus Blight) are very specifically transmitted by leafhoppers or the psyllid species (Garnier, 2001). Fourteen phytoplasma subclasses have been defined, but only two phytoplasmas have so far been named at the genus and species level. A given phytoplasma can infect a broad range of plants, while others are restricted to a single plant species. Importantly, plant pathogenic Mollicutes cannot be controlled chemically today, since the use of antibiotic treatment is forbidden in agriculture. However, research has demonstrated the effective treatment of plant mycoplasma infections via the injection of tetracycline derivatives directly into the bacteria-infected sap distribution channels. Further, it has been established that bacterial antibodies inhibit the growth and metabolism of Mollicutes and such a unique mechanism provides a hopeful approach for future control of these agents in plants (Garnier, 1997).

According to Bove (1997) all three of the currently known, plant pathogenic Spiroplasmas are restricted to the phloem sieve tubes of the infected plants and are transmitted from plant by various phloem feeding leafhopper vectors, within which the Spiroplasmas multiply. Close to fifty other Spiroplasma species or proposed species have been discovered, but are only poorly understood. Without an obvious commercial impetus to determine their cause and effect in plant disease, little research effort or money has been

allocated.

We do know that insects are particularly rich host sources for Spiroplasmas. Some insect-derived Spiroplasmas double as insect pathogens as well. Two Spiroplasma species, *S. melliferum* and *S. apis* are highly pathogenic to honey bees. These species cross the insect-gut barrier and reach the insect hemolymph, where they multiply abundantly and eventually kill the bee, seriously degrading beehive populations, thereby adding a commercial research impetus. *Spiroplasma floricola* is the agent of "lethargy disease"of European cockchafer, i.e. may-bugs. *Spiroplasma poulsonii* infects the neo-tropical species of Drosophila fruit flies, can be readily transmitted, and kills the male progeny of an infected female fly, hence the name "sex ratio Spiroplasma".

Many insect-derived Spiroplasmas can also be found on plants, and in particular, flower petal surfaces. For instance, *S. apis* was cultured from the surfaces of flowers growing in the vicinity of affected beehives. This suggests that the plant-surface abiding Spiroplasmas are deposited on these surfaces by disease-infected or contaminated insects. As such, plant-coating Spiroplasmas may be a novel wild card in future disease research.

Most insect dwelling Spiroplasmas are not insect pathogenic and are often restricted to the gut. These may be regarded as mutualists or incidental commensals most likely deserving little economic research interest. However, recent, ongoing primary investigative Spiroplasma research has uncovered numerous diverse Spiroplasma species in virtually every variety of insect studied to date. This is despite Spiroplasma's fastidious nature and stealth abilities of virtually all the mycoplasmas. This stealth ability has undoubtedly inhibited discovery to date. Only through the use of DNA probes and polymer chain reaction amplification of the archetypical mycoplasma 16S rRNA gene sequence has science finally enabled definitive Spiroplasma identification. To date, ticks, biting midges (sand flies or no-see-ums), mosquitoes, fruit, deer and horse flies, dragonflies, honeybees, leafhoppers, beetles, wasps, all have been identified as potential Spiroplasma carriers (First Internet Conference on Phytopathogenic Mollicutes, Invited Lecture, S*piroplasma* Taxonomy, 2001). Some harbor multiple species (Vazeille-Falcoz, 1997).

Interestingly, French researcher Chastel (1987) found a total of 23 Spiroplasma strains isolated from four species of mosquitoes. Chastel determined that some Spiroplasmas were isolated only from female mosquitoes and only during the months of June and July, attendant with an unidentified virus isolated from three mosquito pools, one pool of which yielded Spiroplasmas. However, specific Spiroplasma viruses as bacteriaphage were not detected. Interestingly, some of the Spiroplasma species found were pathogenic to the mosquitoes and were proposed as a novel but natural mosquito control vector.

The role of Spiroplasmas in mammals is only poorly understood. Strain A56 of the honeybee pathogen *Spiroplasma melliferum* was able to survive up to nine months in intracerebrally inoculated mice. *Melliferum* was associated with a significant runting syndrome and an increase incidence of mortality and neurological symptoms generally

without the appearance of antibody (Chastel, 1991).

In 1992, Chastel further demonstrated that a tabanid (horse fly) Spiroplasma *spp*. produced in vitro cultivation at 37 degrees C, persisted in suckling mice following intracerebral inoculation. This particular *S. spp*. was capable of multiplication and had persistence in mice for 6 successive passages, without the production of specific antibody by the host. Apparently this was the first report of a Spiroplasma from a common flying haematophagous (blood-sucking) arthropod shown to produce persistent infection of a mammal. Importantly, this correlates well with the lack of immune response to Spiroplasmas in small mammals and seems to mimic the well-known immunological tolerance of TSE-infected animals.

Of the three known tick-derived species of Spiroplasmas, none is tick pathogenic. No documented natural adverse reaction has been found within the infected tick host itself nor the tick's normal and natural host, rabbits. However, *Spiroplasma mirum* obtained from Georgia, USA rabbit ticks (*Haemaphysalis leporispalustris*) has been determined to be dosage-dependant, pathogenic to small vertebrates (chick embryo, new-born rodents, and adult rabbits) upon experimental inoculation. Low dose *S. mirum* strain SMCA induces high incidence of cataracts in newborn rodents. Under higher doses of strain *S. mirum* GT-48, no cataracts are observed, but fatal encephalitis or septicemia ensues. Incidentally, this particular source rabbit tick, *Haemaphysalis leporispalustris*, has been implicated as a host of *Borrelia burgdorferi,* the lyme disease spirochete bacteria and the Rocky Mountain Spotted Fever causal agent, the bacteria *Rickettsia rickettsii*.

While no known natural infection of chicks or rodents with SMCA or GT-48 has been documented, its presences within a rabbit tick host suggests that rabbits or other vertebrates preyed upon by these ticks may be logically assumed to contain naturally reproducing Spiroplasma populations. The normal relationship of Spiroplasma to higher vertebrates is virtually unexplored. Undoubtedly, studies on pathogenicity of Spiroplasmas have entered a new and important era (Bove 1997).

Several of the listed potential Spiroplasma carriers, particularly the flies, ticks, mosquitoes and midges are also recognized carriers of other trans-variant vertebrate pathogens. Besides these known and identified disease vectors, quite possibility each of hundreds of thousands of susceptible native insect species may contain one or more of their own individual species of species-specific Spiroplasma or mycoplasma, many of which could conceivably be pathogenic for vertebrates. Are Spiroplasma a pathological risk to vertebrates? A review of the findings derived from Part I: "A Logical Causative Agent" is appropriate.

Several species or subspecies of Spiroplasma have a distinct vertebrate neuronal pathogenicity. Elizan, et al (1972) found that mice inoculated with *Spiroplasma mirum* (SMCA strain) developed prominent microcystic encephalitis within deep gray matter with locally prominent astrocytes. Tully (1982) inoculated suckling rats with either

Spiroplasma mirum GT-48 or SMCA and found that a fatal dose approached 10^9 organisms for SMCA, but that lesser dose of 10^7 to 10^8 organisms showed a high incidence of ocular cataracts. Utilizing rabbit-tick-derived *Spiroplasma mirum* strain, GT-48, Tully, et al (1984) intracerebrally inoculated one-day-old neonatal rats. Only two out of ninety rats receiving the 300-organism dose survived more than 14 days, apparently a lethal dose of GT-48 is easily attained. In conjunction with Tully (1984), Bastian (1984) evaluated infected brain material of GT-48 inoculated rats. Histopathology at 14 days post-intracranial inoculation revealed microcystic encephalitis with Spiroplasmas recognized as filaments, crescents and membrane blebs.

Surprisingly, at and after 25 days post-inoculation, Bastian (1984) noted that electron microscopy showed little inflammation, some neuronal vacuolization, but widespread dilation of neuronal processes. More incredibly, he noted a visually undetectable existence of Spiroplasma organisms, undetectable by microscopy despite significant and measurable assay titers. Continuing in 1987, Bastian demonstrated that intra-peritoneal or subcutaneous inoculation with the vertebrate virulent GT-48 strain of *S. mirum* alleviated short-term mortality but produced alopecia (localized hair loss) and a reduction in body weight. A significant development of cataracts (15 out of 38 rats) both unilateral and bilateral appeared in contrast to prior studies where cataracts were not found with the GT-48 strain. Undoubtedly, *S. mirum* derived from ticks is vertebrate neuropathic.

NOVEL TSE TRANSMISSION VECTORS

Most TSE disease studies have focused upon the presence of one or more of the several TSE disease symptoms, most particularly, the present of proteinase K resistant abnormal prion protein. Failing to find sufficient evidence of transmission among earlier disease studies, several researchers have attempted to find unrecognized TSE disease vectors. An early documented attempt to transmit scrapie by nematodes was a failure (Fitzsimmons & Pattison, 1968).

Upon the suggestion of Icelandic scrapie researcher Sigurdur Sigurdarson, Wisniewski, et al (1996) conducted a pivotal TSE transmission study, collecting 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. Interestingly, ten of 71 mice developed clinical scrapie after inoculation either intra-peritoneally with ground hay mite suspensions (4 of 10) or intracerebrally with the centrifuged supernatant (6 of 10) derived from two of the scrapie infected farms. Diagnosis was confirmed by immunobloting of the diseased mouse brains for abnormal prion protein (PrP^{sc}). Five of the ten positives were derived from a single farm (Farm 1), while three were derived from a second farm.

Mite preparations from Farm 1 were then subjected to proteinase-K, Western Blot determination for abnormal proteinase-K (PK) resistant protein (PK^{res} or PrP^{sc}). Although

no reactions were found under initial mite homogenate concentrations, a 200-fold concentration of Farm 1 mite sample revealed positive PrP^{sc} staining via the non-ovid derived, 3F4 monoclonal antibody. The 3F4 antibody to TSE abnormal prions is not created from sheep tissues. As extensive measures were taken to prevent contamination, Wisniewski, et al (1996) concluded that Icelandic hay mites were potentially a self-sustaining reservoir for the Transmissible Spongiform causal agent although not necessarily a specific sheep scrapie version. The reaction of Farm 1 concentrated homogenate to a non-scrapie derived antibody suggested a common TSE agent present in the mites, perhaps one bridging between scrapie and other TSEs, but not specifically derived from an ovid source.

Co-author Rubenstein (1998) elaborated on the earlier Wisniewski notations. Western blot profiles of Farm 1 positive TSE mouse brains seemingly defined two potential scrapie (or other TSE) strains via three dissimilar-intensity protein bands of 20 to 28 kDa after standard proteinase-K treatment. The 200-fold concentration sample yielded protein bands dissimilar from the typical ME7 or 263K scrapie test strains previously used in the lab. Standard (PK) treatment yielded three 30-33 kDa PK resistant bands, while harsh PK treatment produced a major 26-28 kDa band and several others with significantly lesser intensity. The harsh PK treatment sample was strongly stained with the 3F4 monoclonal antibody, which will not react with sheep-derived PrP^{sc}, but was, in turn, not stained by 7G5 hamster PrP^{sc} sheep scrapie antibody, hence theorically precluding a field or lab scrapie contamination event. This further evidence suggested a source of reaction (or contamination) potentially derived only from a feline or human source (the antibody source of 3F4), but neither group were deemed to have any significant likelihood of contributing the positive result. Hence an unknown TSE source or agent, which can replicate in the mites, was present on Farm 1.

Additionally co-author, Carp, (2000) also used the hay mite preparations from the same five Icelandic farms. Concerned with contamination, Carp subjected mouse brain isolates from the initially positive mice (see Rubenstein, 1998 above) to multiple same-host passages to confirm virulence and compare results with known lab scrapie strains. Using various combinations of "*Sinc*" gene inbred mice (*Sinc* determines scrapie incubation period, Dickinson & Meikle, 1969), three of the four primary mouse isolate passages into the same host species (*Sinc* mice) led to shortened incubation periods and the anticipated clinical symptoms as could be predicted from established scrapie passage recognition characteristics. These results further demonstrated PK-resistant protein bands similar to the ME7 scrapie strain (that found in Icelandic scrapie herds), yet differing in passage incubation periods. 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, without motor nerve dysfunction, yet much more pronounced post-mortem brain tissue vacoulation. While isolates 1 to 3 were sufficiently different from known strains via Sinc incubation periods, they were similar enough to be determined as a probable scrapie reaction, however, the unusual characteristics of isolate 4 did not match any known scrapie strain. From this data, Carp concluded that the results

could not have resulted from field or lab contamination, and in the case of isolate 4, a new and unique TSE strain or agent may be present resident in the mites.

Critical to the importance of the Icelandic mite-TSE studies is the type of mites recovered. The various species found in the pastures in order of abundance were as follows, with the vast majority of mites belonging to the mite sub-family Astigmata of the Family Acaridae, Order Acari:

Scientific Name:	**Common Names:**
Lepidoglyphus destructor	Storage mite
Acarus farris	Grain mite. Cheese mite, Forage mite, Flour mite
Tyrophagus longior	Mold mite, Cheese mite, Copra mite
Cheyletus eruditus	Cannibal mite, Hunting mite, Predatory mite
Tydeus interruptus	Pear-shaped mite, Mouse mite, Tydeid mite
Tarsonemus spp.	Glossy grain mite, Glossy mite

Only the *Cheyletus* mites, found in low quantities, can be described as a direct animal (insect) protein digester, the remainder are plant matter digesters.

Additional research has briefly explored the relationship of TSE with the insect world. Post (1999) approached the potential transmissibility of scrapie agent via a two-pronged approach: 1) by feeding scrapie-infected hamster brain tissue to Sarcophaga (flesh or carrion fly) larvae and 2) exposing 200 mites (predominately orbatid "beetle", "moss" or "litter" mites) to infected hamster brain in a glass bottle for ten days.

In the first study, *S. carnaria* larvae were fed 236K strain scrapie-infected hamster brain or alternatively, healthy control brain. After two days, PK-resistant proteins (PK^{res}), as determined by Western Blot, were present in the dissected scrapie-fed larvae but diminished with time, suggesting fecal evacuation. Dead larvae still possessed detectable PK^{res} 14 day's post-mortem. Additionally, eight hamsters were tube-fed the inner organs of scrapie-infected larvae, while four hamsters orally received *S. carnaria* pupae harvested 10 days after a scrapie brain meal. Six of the eight larvae-fed hamsters developed clinical signs of scrapie with five testing positive for PK^{res} via PK digestion and Western Blot. Two of the four pupae fed hamster developed clinical scrapie symptoms while three tested positive for PK^{res}. Post, et al, speculated that *S. carnaria* had accumulated and conserved PrP^{sc}, but would not speculate as to infectivity replication.

In the second Post study, 60 harvested mites were dissolved in mild salt solution and given orally to two hamsters. Neither developed scrapie symptoms nor PK^{res}. Post concluded that too low a mite dose was given, accounting for the failure to transmit.

DISCUSSION

Significantly, at least two research studies have identified a noteworthy role for insects in the potential transmission of TSE disease. Both research efforts attempted to implicate the conservation of theorically pathogenic PrP^{sc} within the insect body. Certainly that may have been the case in the Post Sarcophaga study where the short time frame of study could seemingly preclude significant agent reproduction and may just present agent conservation. However, the virulence of infection compared with the dosage administered suggests otherwise. One can assume that either the 236K agent is extremely resistant to larvae gut protein breakdown and is highly virulent (potentially both), or it was reproducing independent of the larvae, albeit a low rates within the Sarcophaga larvae. As such, the study is inconclusive. The insect gut environment cannot be regarded as a favorable reproduction ground or a potential source environment for the creation of mammalian prion protein. Mammalian prion protein is foreign to insect proteins.

The mite study is even more problematic. Is the agent just resident in the mites as conserved PrP^{sc} derived from a shedding host and simply indigested by the mites, or are the mites harboring an agent capable of reproduction? Is the mite acting as a non-affected host or a potential disease vector? Either process would conceivably be capable of disease transmission.

The collection procedure for the mites is entirely crucial to a disease propagation theory. The mite assemblage, being collected well after the presence of scrapie-infected sheep in the fields (over one year), and well after the normally short life span of the individual mite hosts and species, lends exceptional credence to the assumption of agent reproduction within the mite corpus, both individually and communally, being subsequently passed into the mite offspring. While some might argue that the mites were simply scouring available residual "prionic" material from the soil previously shed by the infected sheep, the abundance and species of mites precludes such an assumption. These particular mites are predominately astigmatid "stored feed" mites. As such they are typically found in abundance in damp baled hay, and other stored forage or grain environments. These types of mites would not be seeking out or ingesting sheep detritus, yet they have acquired the scrapie sheep agent of infectivity by an unknown assimilation methodology.

Others might say that the mites are creating new, abnormal infectious prions or perhaps passing down the same residual field prions to successive offspring, generation after generation. But, for a mite to be able to reproduce an infective "sheep" prion in a mite corpus environment belies logic. The building blocks for mammalian prions simply do not exist within an insect host to create infective mammalian prions.

The argument that the same old residual abnormal prionic material is passing through multiple generations of mites also seems quite far-fetched under the severe conditions of insect to offspring dilution and the biological necessities of active growth and transport to create the separate and distinct bodies of the new offspring.

The more logical conclusion is that the agent itself can reproduce within the diverse and seemingly adverse environment of the mite body, or perhaps within other creatures of the particular insect assemblage of the Order Acari (also the taxonomic home of rabbit ticks). More reasonably, the infective agent can be passed to its offspring and can be passed to other potential hosts who come into contact with, are bitten by, or perhaps ingest the insect host. Such a mechanism demands that a more rational pathogenic agent to be present than the currently popular prion protein theory. Quite plausibly, the capability of agent reproduction within the mite's highly divergent biological systemia is strong evidence of the truly independent nature of the TSE causal agent. An agent that is self-replacing and fully independent of its host, although perhaps opportunistic in its capabilities.

The possibility of TSE infectivity in *S. carnaria* correlates in an fascinating fashion with the postulates of Purdy (1998) in which he suggests that a 'systemic' pour-on formulation of an organo-phosphorus warblecide (phosmet) during the 1980s initiated the, 'new strain' modification of the CNS prion protein (PrP) causing the UK's bovine spongiform encephalopathy (BSE) epidemic. Summarily, in an attempt to eradicate the warble fly, *(Hypoderma bovis* and *Hypoderma lineatum*) a fly with a muscle-eating burrowing larva, the British government mandated the use of pour-on phosmet to limit the spread of fly damage to cattle herds. The incidence of BSE in British cattle herds closely correlates with the use of phosmet, which of course mimics the range of the warble fly. But the Post (1999) study offers an alternative. One should consider perhaps that the warble fly is itself a vector of BSE infectivity. *Sarcophaga spp*. and *Hypoderma spp*. seemingly possess similar feeding characteristics and reproduction mannerisms to justify further investigation. If the agent can reproduce in carrion flies, those flies may be the source of recurrent infectivity once introduced.

The compelling facts and overwhelming logic of a reproducible, independent TSE agent hosted within insects is difficult to refute. Albeit, insects and TSE have been of minimal research importance to date, future efforts need to be redirected toward this ostensibly logical and basic epidemiological direction. Certainly all can recognize that insects are present everywhere TSE are found, and in fact, may be selectively more abundant in the particular environments where TSE are now being found.

As the additional bacterial evidence shows, of all the potential agents described in the scientific literature, Spiroplasma, a mycoplasma derived from the Class Mollicutes, retains the distinction of harmonizing with the most known TSE agent characteristics when present in vertebrate animals. Insects are a lucrative host for Spiroplasma, yielding an abundance of potential secondary host and attendant Spiroplasma species. Simple logic demands that additional insect-TSE pathogenic studies must be conducted.

A continuing examination of the implications of insects and TSE will be examined in **Chronic Wasting Disease -** Part IIb "**Case Observations on TSE Transmissibility".**

Part IIb will address the observations of several CWD-infected cervidae herds, possible secondary hosts and their novel interaction with the logical Spiroplasma causal agent.

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