#323

Septic Penetrating Setae/Septic Emboli/Septic Penetrating Setal Emboli, (SPS/SE/SPSE): A Hypothesis to Explain the Pathogenesis of the Mare Reproductive Loss Syndrome

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HYPOTHESIS:

We should carefully consider the possibility that simple Septic Penetration of Setae, with hematogenous spread of the septic materials/introduced septic emboli and or septic fragments of barbed Eastern Tent Caterpillar (ETC) setae /Septic Penetrating Setal Emboli [SPSE] or other materials, with associated bacterial "hitchhikers", is the fundamental underlying pathophysiological mechanism of each of the original four recognized 2001 MRLS syndromes, Early Fetal Loss (EFL, n>1,500) Late Fetal Loss (LFL, n>500), Uveitis (n=50) and Pericarditis (n>50) and the more recently recognized Actinobacillus encephalitis cases (n=3).

I now describe this hypothesis in detail and name it the Septic Pentrating Setae/Septic Emboli/Septic Penetrating Setal Emboli [SPS/SE/SPSE] hypothesis of the Mare Reproductive Loss Syndrome [MRLS].

1/ BACKGROUND:

The pivotal assumptions in this hypothesis are that we have incorrectly evaluated and/or under-estimated the combined effect in horses, including pregnant horses of four (4) key factors now in evidence.

1.1/ Factor 1 is the presumed ability of barbed caterpillar setal fragments to **penetrate and/or migrate** in moving tissues, and the generally low rate of significant adverse responses to caterpillar/setal/bacterial exposure. This hypothesis proposes that, in the field, oral exposure to the caterpillar/barbed setal fragments results in penetration of the oral/intestinal mucous membranes by these setal fragments and the carrying with them into the horse of local commensal bacteria, which in the oral cavity are actinobacillus and/or non-hemolytic streptococci. When experimental exposure occurs via the intestinal route, the bacteria introduced are apparently often Serratia spp, a common caterpillar commensal, or other intestinal bacteria ("bacterial hitchhikers").

1.2/ Factor 2 is the second portion of this hypothesis, namely that these setal fragments, by virtue of their barbed nature, can move in moving tissues, and that a proportion of these

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1.2/ Factor 2 is the second portion of this hypothesis, namely that these setal fragments, by virtue of their barbed nature, can move in moving tissues, and that a proportion of these

fragments, or the bacteria therefrom, may enter blood vessels, and may become septic emboli/septic penetrating setal emboli that spread hematogenously throughout the body of the horse. Alternatively, the materials migrating through the blood stream may simply be septic bacterial emboli.

1.3/ Factor 3 is the apparently enormous relative sensitivity of the pregnant mare to exposure to bacteria introduced into the mare, and possibly into the uterus, from/by/with ETC setae or related bacterial emboli as set forth above, as compared with the non-pregnant horse and also, apparently, most other animals, including humans and, as experimentally demonstrated, the mouse.

1.4/ Factor 4 is the less effective protective anti-bacterial/immune responses in the clinically affected tissues. Bacterial contamination of the fetal fluids is well known to allow rapid bacterial growth and resultant fetal loss. The eye is immunologically privileged and less well protected than many tissues. The brain also has immunological deficits, as highlighted by the EPM syndrome. Alone among the clinically affected tissues, a clear immunological deficit cannot be associated with the pericarditis lesions. It may be that the pericardial lesions relate primarily to the unique central location function of the heart in the circulatory system, and its resultant high level of exposure to septic emboli/septic embolic ETC fragments.

(Note: In most tissues a "lodged" septic entity/setal fragment is readily handled by the immune system and other antibacterial defenses and results in no significant long-term damage. However, the apparent lack of effective anti-bacterial systems in the fetal membranes/fluids apparently leaves the fetus largely unprotected once bacterial contamination/proliferation commences in the fetal fluids, rapidly leading to MRLS).

2/ HEMATOGENOUS ORIGINS OF THE MRLS SYNDROMES:

2.1/ From the clinical cases and experimental work it now appears clear that the initiating event in each of the MRLS associated syndromes is a local bacterial infection of hematogenous origin, with the bacteria initially entering the horse's blood stream at the site of exposure to the caterpillar.

2.2/ The cardiac and encephalitic syndromes cannot be other than hematogenous in origin.

2.3/ Recent analysis of the EFL and LFL suggests that bacterial proliferation is the driving force in these syndromes and that the source of the infective bacteria and indeed the primary events are hematogenous and **not** ascending (i.e., via the cervix) in origin.

2.4/ The unilateral opthalmitis cases are also consistent with and best explained by a hematogenous source. In particular, a number of these cases apparently started with hemorrhage deep in the eye, and the difficulty in treating these cases is also most consistent with a septic hematogenous insult commencing deep in the eye. Additionally, none of the eye lesions were

fluorescein positive, which suggests that no significant corneal damage had occurred, and recent experimental work (Bernard 2002 and 2003) has shown that transient topical exposure to caterpillars fails to reproduce the characteristic MRLS associated eye lesions.

3/ PRELIMINARY SUPPORTING EVIDENCE:

This hypothesis would appear to be supported by experiments in 2002 in which EFL was reproduced in mares by stomach tubing of ETC integuments (Bernard) but not by ECT intestinal tracts. More recent experiments reported by Webb (June 2003) apparently show that orally administered frozen ETC can cause this syndrome, while autoclaved ETC cannot. Similarly, 2002 experiments in pregnant mice suggest that intraperitoneal administration of a homogenate/extract of fresh setae produced resorbtion of fetuses in three of six treated mice, although this experiment could not be repeated in one attempt with frozen ETC setae.

Recent (Jan. 2003) work by Bernard has shown that alcohol treated and presumably bacteriologically sterile ETC also produced a case of EFL, matched by one parallel loss in the positive controls. We understand that this fetus showed micropathological evidence of bacteria, consistent with the proposed central role of bacteria in this syndrome.

Additionally, if this hypothesis were correct it would seem probable that caterpillars other than ETC but with similarly structured setae should produce MRLS or MRLS like syndromes. This possibility may be supported by a reported single abortion (1:4) produced by gypsy moth caterpillars in recent (2002) oral administration experiments by Webb.

4/ CATERPILLAR SETAE AND SETAL MIGRATION:

Caterpillar setae are often barbed, designed to penetrate tissues and well recognized to do so. As such, setae may enter the body by any route, including the intestinal route. Once lodged in a tissue, we assume that setal fragments migrate, the rate of migration depending only on the rate/frequency of movement of the host tissue. These tissue movements serve to "ratchet" the barbed seta along in an entirely random direction through the moving host tissue.

5/ SEPTIC EMBOLI/SEPTIC PENETRATING SETAL EMBOLI/ SEPTIC PENETRATING SETAE:

As well as penetrating the intestinal wall, we must also consider that it is also possible that setal fragments that penetrate blood vessels will create "Septic Penetrating Setal Emboli," which may move rapidly to new and more distant locations in the body. All such setal fragment movements would be passive, secondary to tissue movement and/or blood flow, and all events would be statistically determined. These events presumably occur at some level in all species exposed to ECT, apparently with only occasional substantial adverse health consequences, as indicated by

the relatively low incidence of adverse events in exposed but non-pregnant horses and presumably also other exposed species.

This hypothesis is specifically written to describe/cover two possibilities. While the hypothesis assumes that primary entry into the body caused by the ETC is associated with the barbed setae, the second step, namely hematogenous spread, may be due either to the setal fragment themselves, the septic penetrating setal embolus hypothesis, or due to simple hematogenous spread of bacteria, either individually or as bacterial clumps or emboli. While the author (TT) considers that the clinical characteristics of MRLS to be best and most satisfactorily explained by the septic penetrating setal embolus hypothesis, the second possibility, namely that the primary entry is due to septic setal penetration, most likely of some part of the oral mucous membranes/intestinal wall, but that secondary hematogenous distribution as bacterial emboli are both specifically included in this hypothesis.

However the distribution of the septic material to the fetal membranes occurs, the Pregnant Mare, and especially the Late Pregnant Mare, is apparently highly susceptible to fetal loss from Septic Emboli/Septic Penetrating Setal Emboli.

6/ GENERATING SEPTIC EMBOLI/SEPTIC PENETRATING SETAL EMBOLI: CATERPILLAR SETAE, THEIR PROTECTIVE FUNCTION FOR THE CATERPILLAR AND THE ROLE OF THE INTESTINAL TRACT:

For the purposes of this hypothesis, and with reference to the ETC tubing experiments and MRLS in general, we may view the equine intestinal tract, with its ongoing peristaltic movements, as an ideal organ to propel setal fragments into and through its tissues and, with its network of absorptive blood vessels, as a body system highly likely to yield **septic penetrating setae/septic emboli/septic penetrating setal emboli** following ETC exposure.

The finding that Serratia are associated with both ETC and MRLS and actinobacillus and non-hemolytic streps are oral commensals also focuses attention on the potential role of the ETC in introducing ETC surface bacteria/oral commensals into the blood stream as an integral part of the pathogenesis of MRLS. In this regard, the apparently protective efficacy of muzzling mares observed during the 2002 caterpillar season may suggest considerable importance for the oral route of exposure.

(The ability of hairy caterpillars to cause oral lesions in horses is noted in an un-referenced citation in the 74 edition of Blood and Henderson. The ability of short hair fragments to penetrate human skin is well recognized in hairdressing and dog grooming professions, where short hair fragments can readily penetrate the skin and cause a cutaneous condition known as ----------. personal communication, Nancy De Jarnette, Bennet Williams hair salon and also from the scientific literature).

7/ THE STRUCTURE AND FUNCTION OF CATERPILLAR SETAE:

Little attention has apparently been paid to the fine structure and function of ETC setae and similar caterpillar setae. Caterpillar setae have presumably evolved in a manner calculated to maximize the digestive discomfort or other adverse responses associated with their ingestion, it not being in the interests of the caterpillar to be perceived as being readily edible. While the setae of ETC are apparently clearly distinguishable from poisonous setae, they presumably also serve a protective function for the caterpillar.

We suggest that facilitation of bacterial penetration is a primary defensive/aversive mechanism associated with ETC setae and similar setae in other caterpillars. In this regard, ETC and other caterpillar setae may well have evolved in such a manner that their setal protective mechanism is to facilitate the mechanical penetration of bacteria into animals/caterpillar predators. The bacteria thereby introduced proliferate and serve destructive function broadly similar to the enzymatic toxins of some poisonous caterpillar species (Loniomia). This is, presumably, a highly cost-effective defensive strategy for ETC and related caterpillars, the bacterial pathogens substituting for the poisons/toxins/venoms of other species, at a considerable saving of effort/resources for the caterpillar.

(Note: This ETC setal defense mechanism presumably works best when the dose to the affected animal is relatively high, as it would be on a grams of caterpillar/ grams of bird basis, rather than the much larger caterpillar/horse ratio operative in MRLS. The apparently exceptional sensitivity of the horse to caterpillar setae may speak to very poor or absent defenses against this mode of attack in the equine).

MRLS, as we know it, is therefore simply a manifestation of this ETC defensive mechanism. For reasons not readily apparent, the pregnant mare is apparently exceptionally sensitive to this defensive mechanism of ETC. Non-pregnant horses, however, show a very small level of clinical response (see 2001 data, as indicated by the low rate of ETC response in non-pregnant horses and the sensitivity of the eye and heart syndromes to the reduction in ETC exposure from the 2002 MRLS data).

(Note: On the other hand, our emphasis on the oral route does not rule out other routes of exposure. For example, the inhalation routes, a well-recognized route of caterpillar setal exposure in humans, and might well show a relatively more sensitive dose response curve than the oral route) (note ; these were 2002 comments. From the 2003 season, the inhalation route appears to have been ruled out by the nosebag experiment).

(Note: It is of interest that in 2002 the incidence of MRLS decreased to about 1/3 of the incidence of 2001, but that the incidence of the pericarditis and unilateral uveitis syndromes dropped to close to zero/background. This suggests that it was the exceptionally heavy exposure to ETC in 2001 that resulted in these secondary syndromes appearing and being recognized, and that these syndromes, now recognized as an integral part of the MRLS syndrome, may only be

expected to be clinically observable in areas/years of very heavy ETC exposure. These findings also suggest that the dose-response curve for these ancillary syndromes are shifted to the right relative to the fetal loss syndromes, consistent with the exceptional sensitivity of the pregnant mare to exposure to ETC.)

8/ UNUSUAL SUSCEPTIBILITY TO SEPTIC PENETRATING SETAE/SEPTIC EMBOLI/SEPTIC PENETRATING SETAL EMBOLI, (SPS/SE/SPSE) OF THE PREGNANT MARE, AND ESPECIALLY THE LATE PREGNANT MARE:

8.1/ The Role of Myometrial and General Locomotor Activity:

Penetrating/septic penetrating setal/bacterial emboli lodged in uterine blood vessels of pregnant mares will again begin their "through tissue" migration when the mare moves. In uterine tissues, myometrial movement, either due to the musculature of the myometrium itself or, especially in LFL, the physical activity of both the mare and fetus, will again drive migration of the septic setal fragments/lodged septic emboli. Eventually the septic fragment/septic material will penetrate a fetal membrane.

8.2/ The Role of Fetal Membrane Penetration:

Penetration of the fetal membranes alone may be sufficient to cause fetal death. Experience with amniocentesis suggests that limited aseptic trauma to fetal membranes can produce placental separation and fetal death (Dr. Jim Bowen, personal communication).

Well established clinical experience suggests that very modest bacterial contamination of amniotic fluid can result in rapid bacterial overgrowth, followed by death and expulsion of the fetus within a day or days (Dr. Jim Bowen, personal communication). Based on these data, penetration of the fetal membranes by a single septic setal fragment or septic material could be sufficient to produce EFL or LFL.

Review of our recently acquired LFL (date July 2002) experimental data suggests that bacterial proliferation was a primary event in LFL, apparently occurring prior to signs of fetal distress and fetal death.

(Note: On the other hand, toxicological hypotheses assume that toxic damage to the fetal membranes or the fetus is the primary event, followed by secondary bacterial invasion, a proposed sequence of events apparently inconsistent with the data from our recent (2002) LFL/ETC experimental model). (and also from later work by McDowell and Web, June 2003).

8.3/ The Role of Fetal Size and Movement:

A striking characteristic of experimental MRLS was the speed with which the first experimental LFL losses occurred. We suggest that this is because the Late Fetus presents a large "capture

area" for randomly distributing septic emboli/septic penetrating setal fragments; as such, a Late Fetus is statistically more likely to be "hit" within a given period than a much smaller Early Fetus. Additionally, uterine movements are presumably relatively greater in a mare carrying a Late Fetus, driving the tissue migration of septic emboli and or septic setal fragments lodged in uterine tissue, and ensuring their relatively rapid penetration through a fetal membrane. Together, these factors immediately explain the much more rapid onset of LFL than EFL in the recent (2002 and now 2003) ETC oral administration experimental models.

(NB: The role of movement in onset of LFL could be tested with tocolytic agents. Such agents might also conceivably protect against EFL and LFL).

8.4/ Role of the Mare's Placentation Pattern:

It should also be noted that the fetal/maternal placentation interface of the mare covers virtually the entire surface of the placenta. This placental arrangement may render the mare relatively much more susceptible to this kind of attack than, for example, the bovine, with its compact and localized cotyledonary placentation.

Given the simple nature of this model, it is unclear why we have been unable to reproduce EFL/ LFL with oral gavage of caterpillars in mice. However, Rolands has pointed to the remarkable reproductive inefficiency of the horse family (perissodactyls), and notes that members of this family have suffered extinction at a rate greater than any other placentated mammal. It may well be that the relatively unsophisticated placental structure of the horse renders it much more susceptible to this form of attack than the more evolved placental structures of other mammals. Beyond this, other mechanisms by which mice may be protected after oral gavage also suggest themselves.

(Note: Since this hypothesis was first drafted, preliminary evidence suggestive of ETC associated fetal loss in mice has been created by administering a fresh setal homogenate I/P).

(Testing the Hypothesis: The hypothesis suggests that orally administered "shaved" caterpillars should be essentially inactive, while the "shavings" should retain the MRLS and related activities) (June 2003 note: McDowell and Webb have now arrived at the conclusion that the abortigenic activity is associated with the ETC integument).

8.5/ Role of Delayed Fetal Membrane Penetration:

This hypothesis/mechanism readily explains cases of EFL or LFL occurring at some time after exposure to caterpillars has ceased. Inopportune myometrial location of septic material and or a septic penetrating setal fragment, and/or location in a less mobile area of the myometrium would delay onset of entry/puncture of the fetal membranes, yielding classic EFL or LFL at quite some time after exposure to living ETC had ceased.

8.6/ Lack of Positive Blood Cultures:

The septic penetrating setal embolus model is consistent with the general lack of positive blood cultures from EFL and LFL mares. This is because the blood borne bacterial contamination is carried in discrete quantal packets on individual setal fragments or bacterial emboli.

[Note 2003 the fact that it has been very by difficult to identify positive blood cultures in MRLS mares is much more consistent with the septic penetrating setal embolus hypothesis than with the simple septic embolus hypothesis].

8.7 / The role of micro-anaerobes:

The principal bacterial species involved in field MRLS are actinobacillus and non-hemolytic streptococcal species, both micro-anaerobes. These species presumably proliferate well in the fetal membranes/fluids, the low oxygen tension environment of the fetal fluids favoring their growth above those of other microorganisms.

8.8/ The Need for an "Amplification Factor":

One of the puzzling factors about the pathogenesis of MRLS has been the apparently enormous apparent potency of the caterpillar "factor" on a body weight basis. Caterpillars are very small compared with horses, leading to questions concerning how they could synthesize a sufficient quantity of any toxin to produce clear-cut pharmacology/toxicology by the oral route in a 1,500lb horse. This question has led to suggestions of a required biological amplification step (viral contaminants, fungal overgrowth on frass, etc) to provide the requisite potency/multiplication factor.

The bacterial proliferation step itself described herein obviously provides the requisite biological amplification/ multiplication factor, presumably enabling a single septic penetration, setal or otherwise, of a fetal membrane to rapidly produce EFL or LFL in a 1,500lb mare.

9/ The Uveitis, Pericarditis and Encephalitis cases:

This hypothesis requires that not just mares, but that all horses in central Kentucky exposed to ETC suffer essentially equivalent episodes of septic embolus/setal embolus spread. We propose that the uveitis, pericarditis and encephalitis cases, which occurred across central Kentucky in horses of all ages and genders, are clear evidence of this process at work in a systematic fashion, albeit at a very low level in the entire "at risk" population of horses during the period when MRLS occurred.

(Note the earlier comments concerning the apparently substantially different dose response curves for the fetal loss portions of the syndrome and the uveitis/pericarditis cases. Note also the

apparent loss of these related syndromes in 2002. Nature apparently "threw us" some extra bonus point clues in 2001 that we may not see again in nature for some time).

9.1/ The Uveitis Cases:

We now consider it well established that the cases of uveitis are of hematogenous origin. We propose that the primary hematogenous event is delivery of a septic embolus/septic penetrating setal fragment into the eye. The very low incidence of uveitis observed is presumably related to the relatively low target size and or the low fraction of cardiac output supplying an individual eye.

The strongest argument in favor of the septic penetrating setal embolus portion of this hypothesis is that fact that all incidents of uveitis were single eye. This observation well fits a quantal and entirely random distribution of septic penetrating setal fragments, as compared with hematogenous distribution of bacterial emboli or bacterial clumps or individual bacteria, which is less likely to be discernibly quantal in nature.

With reference to Factor 4, the eye is also an immunologically privileged area; as such, the eye may be particularly susceptible to damage by penetrating septic fragments such as septic setae. Our ability to observe eye pathology associated with MRLS is also most likely due to the relative ease of observation of the eye, the highly significant consequences of eye damage compared with limited local damage in other areas of the body and the possible therapeutic/immunological difficulty of controlling a septic focus that has penetrated/entered deep in the eye).

9.2/ The Pericarditis Cases:

The pericarditis cases presumably represent septic emboli/septic penetrating setal emboli that entered the coronary blood supply, lodged in the blood vessels and then proceeded to migrate through the cardiac muscle. Of all muscles in the body, the heart is the one through which one might expect septic emboli/septic penetrating setal emboli to migrate fastest. Additionally, for every septic emboli/septic penetrating setal emboli that migrated "out" and appeared at the epicardial surface, at least one migrated in the opposite (or other) directions. Presumably a much larger number of subclinical epicarditis cases occurred associated with the MRLS episodes, and resolved spontaneously, as presumably do most incidents of systemic septic setal spread.

[Note: Dr. Clara Fenger, a skilled clinician and internist, reports several incidence of subclinical pericarditis treated on her farm in association with ETC exposure episodes].

(Note: A problem with this hypothesis is that pathologists have reported no evidence of setal tracks in cardiac tissues. Additionally, a careful search should be made for signs of subclinical pericarditis associated with intestinal exposure to ETC. The central role of the heart in the circulatory system and its ongoing mechanical activity may suggest a considerable probability of

transient positive histological and bacteriological culture findings in pericardial fluid associated with ETC exposure).

9.3/ The Encephalitis cases:

Since MRLS was first described three specific cases of actinobacillus encephalitis have been recognized as occurring in the same period. Like the pericarditis cases, these cases are unquestionably hematogenous in origin and occurred in or about the time period as the MRLS ETC.

10/ WHY HORSES?

Early suggestions that ETC were centrally involved in MRLS immediately ran into the problem of the well established harmlessness of ETC. Review of events since May 2001 shows that ETC are extremely harmful to pregnant mares. They can also be harmful to non-pregnant horses if the rate of exposure is high (2001 dose levels), but the rates of damage and death are still hundreds to thousands of times lower than the damage rates in pregnant mares.

It is unclear whether or not the rates of injury from ETC in non-pregnant horses are different from those in other species. However, there is an enormous disparity in size between the caterpillar and the horse, and the fact is that the only ETC associated syndromes so far recognized in any species are all in the horse. This fact may suggest that the horse, pregnant or non-pregnant, is relatively/very poorly protected against the ETC factor producing MRLS and its related syndromes. Consistent with this suggestion, the apparent ease with which mice resisted a relatively 50 times greater oral dose of ETC for up to 12 days, and gained weight in the process, speaks to the apparent ease with which the ETC factor can be resisted in one (some? most?) species.

11/ INTELLECTUAL ECONOMY OF THE HYPOTHESIS:

11.1/ This hypothesis is a modification and simplification of the hypothesis that drove our first mouse setal experiment, which assumed that the setae were introducing a protein toxin that was the primary pathogen. Further reflection, however, suggests that a setal toxin is not necessarily required, as set forth above, and the speed of onset of LFL in the laboratory LFL experiments in pregnant mares also does not suggest a classic catalytic toxic mechanism.

11.2/ This hypothesis does not require or assume the presence with or in the caterpillars of any extra toxins, venoms, viruses, unusual weather patterns, other plant toxins, fungal overgrowth, duck poop, etc. (Which, unfortunately, seems to severely limit its intellectual property potential/ applications).

11.3/ We must also note that a clear characteristic of MRLS has been that no traces, associations or evidence of toxins, viruses, mycotoxins, etc, other than the bacterial species set forth above

have thus far been identified in association with this syndrome, despite very extensive searches for the same.

11.4/ This hypothesis may also explain why no significant hormonal patterns or other clinical chemistry changes have yet been identified in the aborting mares. MRLS, both EFL and LFL, is dependent on the direct seeding of small amounts of bacterial contaminants directly into the fetal membranes. The bacterial proliferation occurs directly in the fetal membranes, followed by bacterial invasion of the fetus, which is then rapidly "slipped".

11.5/ This primary hypothesis is grounded in the well-established physics and mechanics of the movement of barbed fragments through motile soft tissues, and the likelihood of bacterial contamination of such barbed fragments. The secondary subhypothesis assumes that a bacterial embolus behaves approximately similarly. We propose that these hypotheses can account for all of the major characteristics of the five simultaneously occurring MRLS syndromes.

12/ OTHER CATERPILLARS???

If this simple hypothesis is correct, then similar exposure to mechanically equivalent setae from other caterpillar species with equivalent setae, or from any other mechanically equivalent setae should also have the potential to produce MRLS. (Note: This conclusion may or may not be supported by the reported single abortion in four pregnant mares orally administered gypsy moth caterpillars by Webb, although we must also note that Webb has elected to interpret this result as being atypical and non-MRLS related).

[A major problem with this hypothesis that has arisen is that Forest Tent Caterpillars have apparently been unable to reproduce MRLS. It should be noted, however, that recent experimental data seems to show and can be interpreted on the basis that ETC setae easily lose their mechanical integrity, and are, under some circumstances, fragile. Since the Forest Tent Caterpillars were homogenized in New York, frozen and shipped to Lexington for experimental use, it is possible the abortifacient activity/setal integrity was lost during these events, as set forth in the attached memorandum, which is made a part of this document].

RE: ETC Factor Fragility:

Think Tank Members:

Whatever we are dealing with on the outside of the caterpillar is **fragile.** Manu almost lost it in the irradiation expt, starting with 100 g of caterpillars.

Bruce also lost a significant chunk of it in his ETC "pelt" experiment, starting with 50 grams of ETC and probably not manipulating them very much.

Whatever happened in the "homogenization" experiment wiped out essentially all of the abortifacient activity in that experiment.

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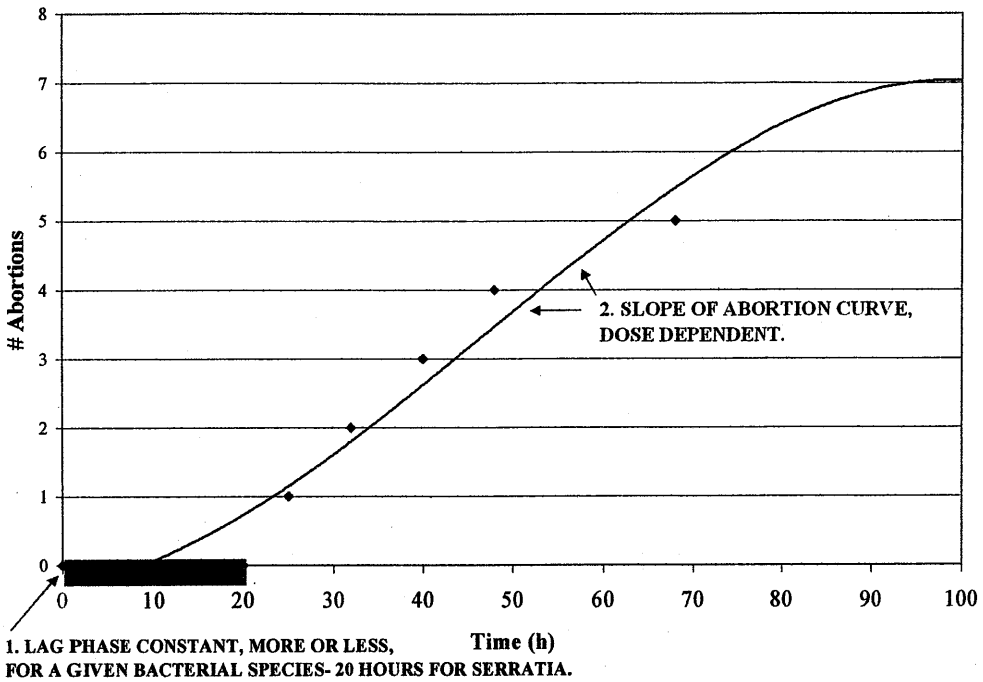
So, starting with high dose controls means that when you are finished manipulating the caterpillars, you may still retain enough activity to produce abortions, and thus an interpretable experiment.

Addendum Number 2: Proposed working hypothesis of MRLS and how it relates to the Septic Penetrating Setae/Septic Emboli/Septic Penetrating Setal Emboli, (SPS/SE/SPSE) hypothesis and the recently completed irradiation experiment.

Figure #3
Proposed Two-Phase Working Hypothesis of MRLS



The author suggests that all the MRLS experiments/data to date may be interpreted to support the simplest possible ETC/ MRLS model as follows:

1/ Step #1: Horse Entry:

The first step is rapid ETC/setal associated entry of Serratia and/or other organisms into the circulatory system and organs of a mare/(horse) (Horse Entry Event) within less than 10 hours of a 100 gram ETC tubing dose. We respectfully suggest that we should test this hypothesis with a small number of high dose experiments in non-pregnant horses to be put down and their organs cultured for Serratia and/or other ETC comensals and for micropathological evidence of same. We propose that this step is associated with the entry of barbed ETC fragments into the tissues of the horse, most likely through the oral and or intestinal mucous membranes.

2/ Step #2: Random Distribution:

The introduced organisms randomly distribute throughout the horse, including the heart, abdominal organs, the eye (singular), the brain and, of course, the uterus.

We suggest that this distribution follows cardiac output. The single eye events suggest that distribution is both random and quantal.

We propose that the data on MRLS are best explained by the hematogenous distribution of septic penetrating setal emboli fragments and or septic bacterial emboli, either or both originating from the original penetration of the horse associated with septic setal fragment(s).

3/ Step #3: Uterine Entry (exponential function points):

Next, Uterine Entry Events from the circulatory system commence within 10 hours or less based on a 100 g ETC dose. The rate at which these Horse Entry and Uterine Entry Events occurs depends on the effective dose of ETC administered, which is experimentally reflected in the mid point slopes of the Accelerated Failure Time (AFT) abortion/survival curves. Entry Events are, of course, not restricted to the uterus, but occur elsewhere in the body, accounting for the eye, heart and recently identified CNS manifestations of MRLS. We also suggest that they may be very marked in the intestinal tract and hepatic portal system, and note that this possibility has not been investigated.

We propose the data on MRLS are best explained by the hematogenous distribution of septic penetrating setal emboli fragments and or septic bacterial emboli, either or both originating from the original penetration of the horse associated with septic setal fragment(s).

4/ Step #4: Bacterial proliferation (lag time):

Bacterial proliferation then occurs in the fetal fluids and abortion follows 20+ hours after the Uterine Entry Event if the organism introduced is Serratia. At least part of the lag time in the model presented above is presumably related to the time taken for the bacteria to proliferate and produce an abortion event.

5/ The mathematical nature of the family of dose response curves:

The shape of the dose response curves and the AFT analysis and projections suggest that the **rate of the abortion response is EXPONENTIALLY RELATED TO THE DOSE OF ETC.** This finding likely explains the exceptionally intense and focused nature of the 2001 MRLS outbreak. Additionally, from the fit to this equation, we can estimate the effective dose of ETC, in grams of fresh intubated ETC units, for any abortion response. We draw the reader's attention to

the potential in 2001 for exceptionally high ETC exposure rates, as suggested by the well-known Henry Murphy water bucket picture.

6/ The Entry Event factor:

The question remains as to the nature of the factor in the ETC that facilitates the Horse Entry and Uterine Entry Events. We suggest that we provisionally call this the ETC Entry Factor (EEF). Economy suggests we provisionally assume that one factor facilitates both the Horse and Uterine Entry Events.

7/ The nature of the ETC Entry Factor is at this time unknown. This author has a marked preference for the septic penetrating setal embolus hypothesis, as outlined in this communication, with a less distinct preference for septic penetrating setae followed by hematogenous distribution of bacterial emboli. The bacterial embolus hypothesis is considered a somewhat less satisfactory hypothesis because the hematogenous distribution of septic penetrating setal fragments immediately explains the very rapid penetration of the uterine tissues found in MRLS and to a lesser extent the penetration of the eye and heart tissues.

Interpretation of the Irradiation Experiment Data: Preliminary evaluation of the partial irradiation data suggests that:

17.1/ The irradiated caterpillars produce fetal loss, but with what appears to be a greatly prolonged (80-hour, by AFT analysis) lag time and considerably reduced efficacy, based on the reduced slope of the abortion/survival curve. The irradiated caterpillars have apparently lost about 99% or more of their ETC Entry Factor activity. Nevertheless, they still have aborted 50% of the mares.

17.2/ This prolonged lag time may be related to the change of bacterial pathogens. The Serratia are no longer apparent, presumably eliminated by irradiation, while bacteria associated with field MRLS have appeared. The ETC Entry Factor now facilitates the entry of different bacterial pathogens into fetal membranes, consistent with the pathogens found in field MRLS. The Entry Factor may have to interact with these new pathogens, which may themselves be slower or less efficient at inducing abortion after an Entry Event. The Entry Factor is apparently an "equal opportunity" Entry Factor. Both of these circumstances may contribute to the longer lag times in the Actinobacillus/Strep based field manifestations of MRLS.

17.3/ Judging by the long list of MRLS pathogens found in nature, which clearly includes Serratia, the Entry Factor would seem to be an "equal opportunity factor," and there is no reason in high dose experiments that entry should be limited to a single penetration event or to a single species of facilitated organism.

17.4/ The change in the slope of the abortion survival curve suggests that events associated with the irradiation have very substantially reduced the inherent efficacy of the ETC Entry Factor.

17.5/ We may also wish to note that the AFT mathematical projection of the time to abortion for 100% (the last abortion) of the irradiated ETC mares is 4,900 hours (~204 days), although the third abortion in the irradiated group arrived this morning, more or less on its mathematical schedule.

18/ Based on these analyses of the tubing experiments, we submit that these experiments have been highly productive and have contributed very substantially to our understanding of MRLS and the critical role of the ETC and the so far unidentified apparently ETC-related Entry Factor in this syndrome. We also submit that the tubing data immediately suggest further simple, rapid and highly "doable" analytical and experimental approaches, and, most importantly, may provide a framework for analysis and interpretation of all ETC experiments.

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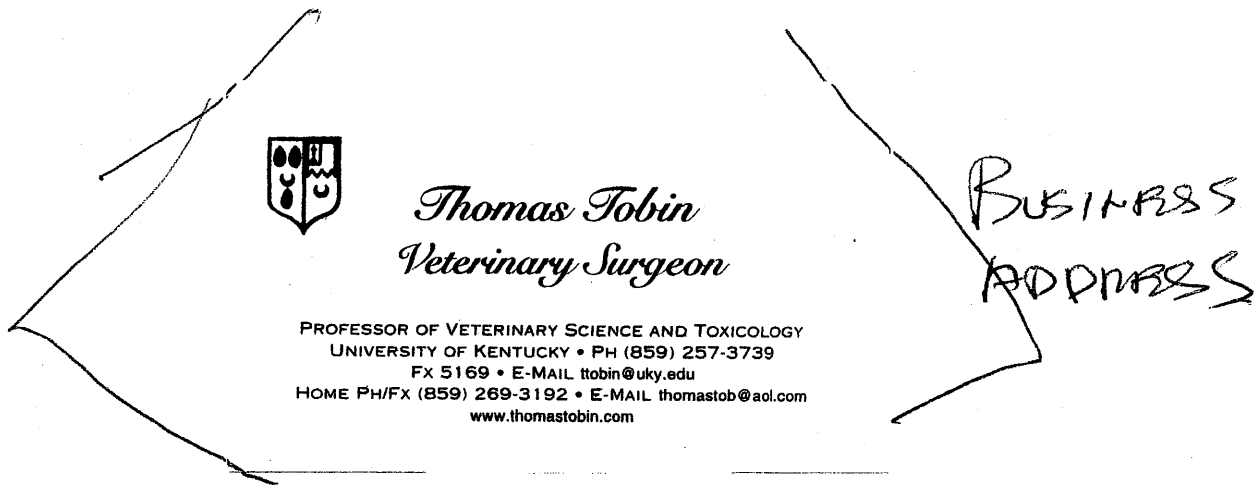

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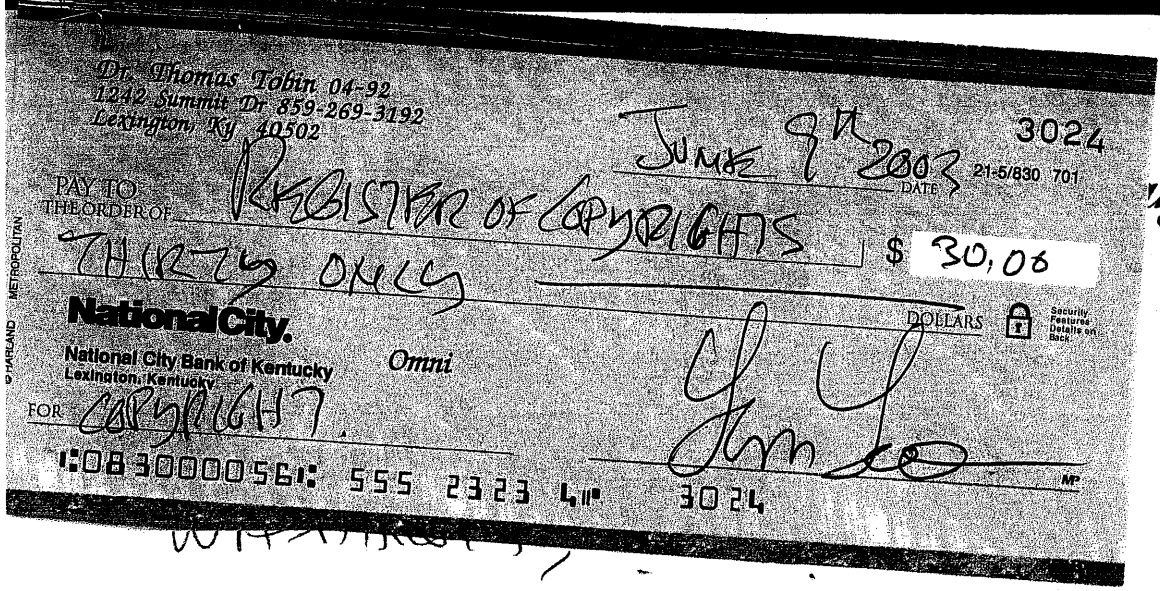

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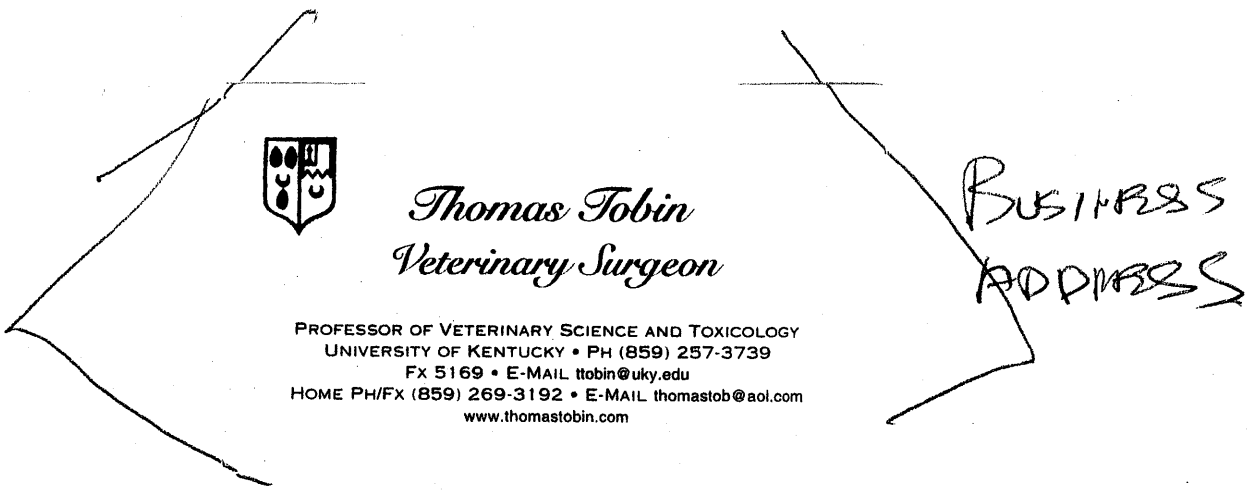

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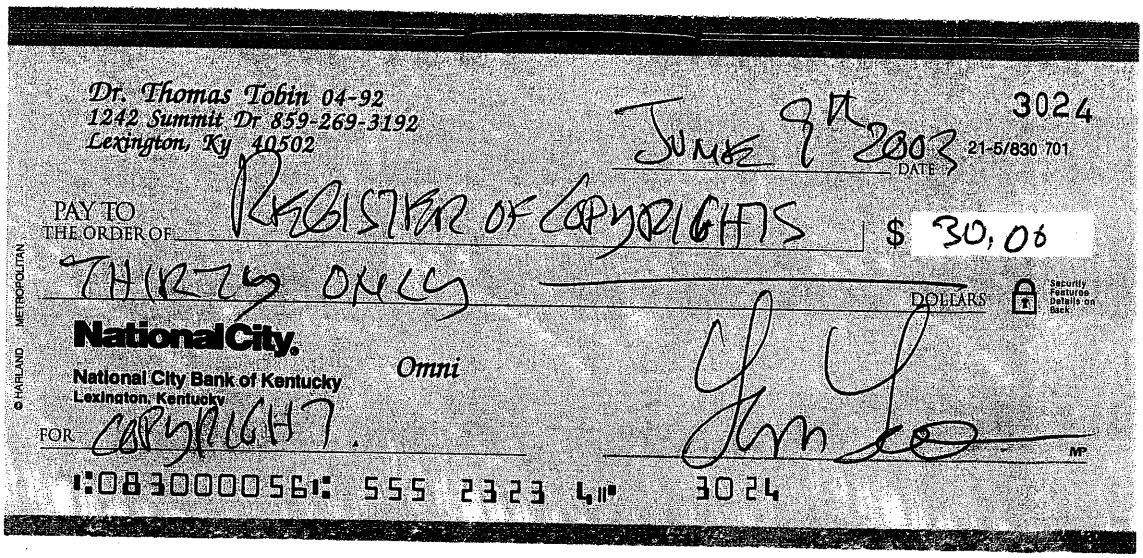

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