UNIVERSITY OF KENTUCKY

Veterinary Science
College of Agriculture
GLUCK EQUINE RESEARCH CENTER
Lexington, KY 40546-0099
(859) 257-4757
www.uky.edu
Fax: (859) 257-8542
Writer's Direct Dial Number

Thursday, April 14, 2005
4:50 PM
By e-mail and surface mail

Dr. Nigel Perkins
Hunter Valley Equine Research Center
AusVet Animal Health Services
PO Box 3180
South Brisbane 4101
Australia

Re: EAFL and MRLS

Dear Dr. Perkins,

I have reviewed your very interesting and well written March 2005 report on Australian Equine Amnionitis and Fetal Loss [EAFL] and the following are my preliminary thoughts and suggestions.

I will open this letter with my summary, go through your report as I see it, and then, for emphasis, close with the summary again.

Summary:

Based on my review of your excellent report, my suggestion would be that you proceed as soon as possible with Caterpillar administration experiments, and see what results. I think the probability of a positive answer with the Caterpillar experiments is very much, in fact enormously, higher than with the possible pennyroyal experiments.

If you have not read our " - Toxicokinetic/Statistical Analysis --" of MRLS in Vet. Therapeutics, Vol 4, #4, Winter 2003 pages 324-338, I suggest that you do so. The paper describes how American Eastern Tent Caterpillars (ETC) produce abortions on dose, on schedule, and on time, and also closely following an unusual mathematical equation. This is no accident, and this model has to be your first line of approach to the problem you have so well described in your report.

Page #3:

Overall Characteristics of the Syndrome:

1/ My overall sense is that this syndrome has many characteristics in common with the Kentucky MRLS syndrome, and my sense would also be to follow up the caterpillar connection as rapidly as possible. I believe this syndrome has many characteristics that suggest caterpillar involvement, as we have found for MRLS, and very few characteristics suggestive of a typical plant toxicity.

2/ ***"Mares show little or no signs of illness prior to abortion"***

One of the characteristics of MRLS that became very apparent as we worked with MRLS and our laboratory models of MRLS was that we saw essentially no clinical signs in the mares whatsoever. Your EAFL syndrome seems to be very similar in this respect. Additionally, in our MRLS models we looked for bacteremia and never found any evidence of bacteremia.

3/ The pathological changes involving ***"the amnion, the amnionic portion of the umbilical chord and inner allantoic surface of the chorioallantois"*** are, to the best of my knowledge, pathological characteristics of MRLS, although as a non-pathologist, I would have to defer to my pathologist colleagues in this area.

Since the MRLS placental pathology represents a unique placentitis and the pathology of the EAFL placentitis is quite similar, this supports suggestions of similar basic mechanisms underlying these syndromes.

4/ The recovery of ***"a range of unusual environmental bacteria".***

One of the interesting characteristics on MRLS is the relatively wide range of bacterial pathogens involved, and you seem to have seen this also in EAFL. In fact, you seem to have pinpointed the role of ***"environmental"*** bacteria much more accurately and specifically than we did in Kentucky. These are apparently what I chose to generically call the "bacterial hitchhikers" in my original analysis of MRLS.

5/ You seem to have clear potential involvement of caterpillars, and your candidate caterpillars apparently have both setal penetration and urticating capabilities.

6/ Based on what I have seen to date, the caterpillars are, in my opinion, prime candidates, and measures to reduce exposure to caterpillars should be recommended/undertaken.

Page #8:

Proposed Experiments:

I suggest you dose pregnant mares with caterpillars:

If my evaluation is correct, and if it is indeed the caterpillars and their setae, then a simple caterpillar intubation experiment will most likely settle the matter of the etiology of this problem and allow you to focus your attention on the caterpillars.

Given the simplicity of the caterpillar experiment, and the wild goose nature of chasing poorly defined immunosuppressive toxins, I strongly recommend pursuing the caterpillars first. One of the reasons we pursued cyanide was that we had a full year to wait for the Eastern Tent Caterpillars to return.

You also need to know that of a number of candidate toxins, including cyanide, were administered to pregnant horses in Kentucky while we were waiting for the ETC to return in 02. None of these administrations, repeat none, zero, zip, nada, produced abortions. On the other hand, as soon as the ETC caterpillars returned in 2002, anybody who could get their hands on caterpillars could abort horses on schedule.

So, caterpillar experiments first, everything else second.

Page # 13, Section 11:

I believe that your description of the bacteria identified from EAFL, their wide range, the fact that they are not usually associated with disease in horses, and that there is no evidence of a "spreading" disease is entirely consistent with a primary caterpillar/setal mechanism for EAFL, just like MRLS.

Page # 13 Section 12:

"It is not yet clear ----- why the bacteria locate in the uterus"

I thought this was very clearly set forth in my proposed setal pathogenesis of MRLS. In MRLS the setal fragments that enter intestinal blood vessels distribute **entirely randomly** through the body, **following cardiac output,** just as drug molecules do. At this point it helps to think like a pharmacokineticist except we are dealing with fragments of setae and not drug molecules.

In MRLS the late fetus/fetal membranes receive an estimated 15 % of cardiac output, maybe more. They are a huge target (capture area), and therefore collect 15% of all distributing setal fragments.

Because of their retained capability to penetrate moving tissues, a lodged setal fragment again continue to move through the moving tissues/fetal membranes, carrying with them the passenger bacteria, your environmental bacteria, my MRLS setal "hitchikers".

These bacteria are obviously environmental bacteria and generally cause no disease/pathology. However, it is the **poor immune responses in the fetus/fetal membranes** that allow these bacteria to proliferate and cause disease/abortions. The late fetus is the largest poorly immunologically protected tissue mass in the mare's body.

So the bacteria **do not "locate"** there. Rather, once they have entered the fetus, their **proliferation proceeds largely unchecked,** and you soon get the resulting abortions, in as little as 30 hours if you dose enough ETC.

Proliferation following setal lodgment in other tissues is prevented by the normal immune responses in these tissues. Exceptions in MRLS seem to be the eye, well known to be immune privileged, and the pericardial fluids and also, to a very minor extent, the brain.

The incidence of MRLS eye and heart lesions is very small We saw these in 01 in Kentucky because of the very large number of horses and caterpillars interacting in 01. It seems your EAFL numbers in Australia are very much smaller that the 30,000 horses exposed to high concentrations of caterpillars in Kentucky in 01.

Page # 14 Paragraph #2:

"Caterpillar hairs":

I believe this paragraph is on the mark. One of the unusual things about our MRLS work was the exceptional speed with which abortions occurred after intubation of caterpillars, which we believe is entirely consistent with and can only be satisfactorily explained by rapid hematogenous distribution of setal fragments, and their retained tissue penetration capabilities.

It will be very interesting to see whether or not the Processionary caterpillars will produce abortions very rapidly, essentially on demand, and on time, as the Eastern Tent Caterpillars did in our hands.

I should also note that one of the predictions of the setal hypothesis of MRLS, presented in the very first drafts of this hypothesis, was that MRLS type abortions should not be expected to be restricted to just Eastern Tent Caterpillars. Tissue penetration was assumed to be principally a mechanical property of the barbed setal fragments and any mechanically equivalent setal fragment or indeed structure should do the trick.

Which leads to one simple suggestion; have you looked carefully at the setal fragments of your processionary caterpillars?? Are the setal fragments barbed, such as to permit tissue migration? Are some of them very small and fine? This is not a very difficult thing to do.

Page # 14, Paragraph #3:

If I may respectfully make a suggestion, it makes very little sense to invoke an immunosuppressive toxin in pennyroyal, because, if my thinking is correct, the fetal membranes have greatly reduced immuno-competence, and there will be no additive effect of the postulated immunosuppressive toxins of pennyroyal. I would assign this hypothesis very much secondary status.

Summary:

My suggestion would be to proceed as soon as possible with Caterpillar administration experiments, and see what results. I think the probability of a positive answer with the Caterpillar experiments is very much, in fact enormously higher than with the possible pennyroyal experiments.

If you have not read our " - Toxicokinetic/Statistical Analysis --" of MRLS in Vet. Therapeutics, Vol 4, #4, Winter 2003 pages 324-338, I suggest that you do so. The paper describes how ETC will produce abortions on dose, on schedule, and on time, closely following an unusual mathematical equation. This was no accident, and this model has to be your first line of approach to the problem you have so well described in your report.

Yours sincerely,

Thomas Tobin
MVB, MSc, PhD, MRCVS, DABT
Professor of Veterinary Science
Professor Graduate Center for Toxicology
Attachments
1/ Page 75 MRLS workshop
2/ Vet. Therapeutics, Vol 4, #4, Winter 2003, pages 324-338
3/ http://www.jarvm.com/articles/Vol2Iss2/TOBINJARVMVol2No2.pdf
4/ www.thomastobin.com