Maxwell H. Gluck Equine Research Center Faculty Publications

Maxwell H. Gluck Equine Research Center

Winter 2003

The Mare Reproductive Loss Syndrome and the Eastern Tent Caterpillar: A Toxicokinetic/Statistical Analysis With Clinical, Epidemiologic, and Mechanistic Implications

Manu Sebastian
University of Kentucky

Marie G. Gantz
University of Kentucky

Thomas Tobin
University of Kentucky, ttobin@uky.edu

J. Daniel Harkins
University of Kentucky

Jeffrey M. Bosken
University of Kentucky

Follow this and additional works at: https://uknowledge.uky.edu/gerc_facpub

Part of the Large or Food Animal and Equine Medicine Commons
See next page for additional authors
Right click to open a feedback form in a new tab to let us know how this document benefits you.

The Mare Reproductive Loss Syndrome and the Eastern Tent Caterpillar: A Toxicokinetic/Statistical Analysis With Clinical, Epidemiologic, and Mechanistic Implications

Notes/Citation Information

Authors

Manu Sebastian, Marie G. Gantz, Thomas Tobin, J. Daniel Harkins, Jeffrey M. Bosken, Charlie Hughes, Lenn R. Harrison, William V. Bernard, Dana L. Richter, and Terrence D. Fitzgerald

The Mare Reproductive Loss Syndrome and the Eastern Tent Caterpillar: A Toxicokinetic/Statistical Analysis with Clinical, Epidemiologic, and Mechanistic Implications*

Manu Sebastian, DVM, MS[a]
Marie G. Gantz, MS[b]
Thomas Tobin, MVB, MS, PhD, MRCVS, DABT[c]
J. Daniel Harkins, DVM, PhD[c]
Jeffrey M. Bosken, BS[c]
Charlie Hughes, MS[c]
Lenn R. Harrison, VMD[a]
William V. Bernard, DVM, DACVIM[d]
Dana L. Richter, PhD[e]
Terrence D. Fitzgerald, MS, PhD[f]

[a]*Livestock Disease Diagnostic Center*
[b]*Department of Statistics*
[c]*Maxwell H. Gluck Equine Research Center*
University of Kentucky
Lexington, KY 40546

[d]*Rood and Riddle Equine Hospital*
2150 Georgetown Road
Lexington, KY 40511

[e]*School of Forest Resources & Environmental Science*
Michigan Technological University
1400 Townsend Drive
Houghton, MI 49311

[f]*Department of Biological Sciences*
SUNY Cortland
PO Box 2000
Cortland, NY 13045

■ ABSTRACT

During 2001, central Kentucky experienced acute transient epidemics of early and late fetal losses, pericarditis, and unilateral endophthalmitis, collectively referred to as mare reproductive loss syndrome (MRLS). A toxicokinetic/statistical analysis of experimental and field MRLS data was conducted using accelerated failure time (AFT) analysis of abortions following administration of Eastern tent caterpillars (ETCs; 100 or 50 g/day or 100 g of irradiated caterpillars/day) to late-term pregnant mares. In addition, 2001 late-term fetal loss field data were used in the analysis. Experimental data were fitted by AFT analysis at a high ($P < .0001$) significance. Times to first

M. Sebastian, M. G. Gantz, T. Tobin, J. D. Harkins, J. M. Bosken, C. Hughes,
L. R. Harrison, W. V. Bernard, D. L. Richter, and T. D. Fitzgerald

abortion ("lag time") and abortion rates were dose dependent. Lag times decreased and abortion rates increased exponentially with dose. Calculated dose × response data curves allow interpretation of abortion data in terms of "intubated ETC equivalents." Analysis suggested that field exposure to ETCs in 2001 in central Kentucky commenced on approximately April 27, was initially equivalent to approximately 5 g of intubated ETCs/day, and increased to approximately 30 g/day at the outbreak peak. This analysis accounts for many aspects of the epidemiology, clinical presentations, and manifestations of MRLS. It allows quantitative interpretation of experimental and field MRLS data and has implications for the basic mechanisms underlying MRLS. The results support suggestions that MRLS is caused by exposure to or ingestion of ETCs. The results also show that high levels of ETC exposure produce intense, focused outbreaks of MRLS, closely linked in time and place to dispersing ETCs, as occurred in central Kentucky in 2001. With less intense exposure, lag time is longer and abortions tend to spread out over time and may occur out of phase with ETC exposure, obscuring both diagnosis of this syndrome and the role of the caterpillars.

■ INTRODUCTION

During 2001, central Kentucky experienced acute transient epidemics of early (EFL) and late fetal losses (LFL), pericarditis, and unilateral endophthalmitis, collectively referred to as mare reproductive loss syndrome (MRLS). The occurrence of EFL was first recorded on the morning of April 26, increased during the last week of April in mares with 30- to 100-day-old fetuses, peaked on May 4, and declined rapidly thereafter, ultimately totaling approximately 2,500 cases.[1] The largest number of EFL cases occurred in mares that had conceived in February and early March. Approximately 17% of

the 21,000 or more pregnant thoroughbred broodmares in central Kentucky lost their foals due in 2001 or 2002.[2] Whatever the mechanism of EFL and LFL, the pregnant broodmare (or more likely the equine fetus) is especially susceptible. The 17% fetal loss rate is in sharp contrast with the relatively low rate of MRLS-related pathology in nonpregnant horses.

Instances of LFLs were first identified soon after April 26 and followed a broadly similar time course, involving more than 650 cases. The placentas from these mares that experienced LFL were mildly edematous; however, most were of normal size and weight. Some mares presented foals normally but showed evidence of amnionitis with yellowish discoloration and edema of the placental membranes. Pathophysiologically, there was an intense placentitis associated with thickened placental membranes. Funisitis associated with the amniotic section of the umbilical cords of these neonates was observed with ecchymotic and petechial hemorrhage on the surface of the cords. Microbiologically, the consistent recovery of α-hemolytic streptococci and *Actinobacillus* spp from umbilical tissue, lungs, and placentas of affected animals was almost a hallmark for defining the clinical entity of LFL MRLS.[3]

Coincident with the 2001 MRLS epidemics, small numbers of horses from the population of approximately 30,000 horses in central Kentucky exhibited panophthalmitis (approximately 30 cases) or pericarditis (approximately 60 cases). Both of these syndromes are now considered part of the MRLS entity. Fluid drained from the pericardial sacs of two-thirds of the horses with pericarditis was characterized as sterile exudates, whereas one-third had septic exudates containing *Actinobacillus equuli*, *Streptococcus* spp, *Pasteurella multocida*, *Staphylococcus aureus*, *Acinetobacter*, or *Pseudomonas*.[4] The endophthalmitis was always uni-

lateral and invariably progressed to blindness despite intensive therapy.[5] Whatever the nature of the factor that produced the MRLS outbreaks, the nonpregnant horse appears to be about 50 to 100 times less susceptible than are 30- to 100-day-old fetuses. This same syndrome was repeated in 2002, with fewer EFLs (approximately 500) and LFLs (approximately 165)[6] and similarly reduced numbers of unilateral panophthalmitis (n = 6) and pericarditis cases (n = 9). In 2003, there were few if any cases of MRLS, and no cases of panophthalmitis or pericarditis were reported.[a]

When MRLS was first observed in 2001, a spectrum of possible causes was considered. An infectious agent was ruled out early by virtue of intensive virologic investigations, the essentially complete lack of clinical symptoms other than abortion in affected mares, and the apparent point-source onset of the syndrome. Nitrate/nitrite toxicosis was also discounted early, since all equine samples tested were negative for these inorganic ions. Similarly, contemporary pasture samples, some from heavily fertilized fields, were also negative for nitrates/nitrites. Affected mares did not have prolonged gestations as seen with fescue toxicosis, and the placentas from these mares were intensely more inflamed than is seen with fescue toxicosis, so ergot alkaloids were also soon discounted as a possible cause.[7] Phytoestrogens, chemical toxins, mycotoxins (including estrogenic mycotoxins), and cyanide associated with black cherry trees or Eastern tent caterpillars (ETCs) were all eventually downgraded as possible causes of MRLS.[8–11]

Concurrent with the 2001 EFL and LFL epidemic was a local population explosion of ETCs *(Malacosoma americanum)*, with large numbers dispersing in pastures and elsewhere during the period when EFLs and LFLs peaked. Contemporary field evaluations pointing to strong time and location correlations between EFLs and the presence of ETCs, and rigorous epidemiologic surveys strongly suggested that exposure to ETCs played a critical role in the syndrome.[12,13] These associations were rapidly confirmed by various experimental approaches when ETCs next appeared in central Kentucky during the spring of 2002.[14–16]

This communication presents a retrospective toxicokinetic/statistical analysis of experimental data generated from field data during the 2001 MRLS outbreak and studies of ETC populations conducted during 2002 and 2003. Both traditional linear regression and a more appropriate survival analysis were used to analyze the data. The accelerated failure time (AFT) survival model was used because AFT is specifically designed to relate events to the time at which they occur. The ramification of combining experimental data from multiple years is that there could be differences in conditions among years that are not accounted for in the model. The prospective experimental data were used to create the AFT model, whereas the retrospective field data were examined post hoc in light of the AFT model.

The AFT analysis has not been applied previously in equine toxicology. This analysis highlights the epidemiology, clinical presentations, and manifestations of MRLS and sheds light on the basic toxicologic and pathologic mechanisms underlying MRLS. It also allows for quantitative interpretation of experimental results from ETC–induced MRLS.

■ MATERIALS AND METHODS

Studies conducted during 2002 involved dosing of six mares in late pregnancy with 50 g of ETCs per day for 10 days by stomach tube (Table 1).[16] ETCs were obtained from a

M. Sebastian, M. G. Gantz, T. Tobin, J. D. Harkins, J. M. Bosken, C. Hughes,
L. R. Harrison, W. V. Bernard, D. L. Richter, and T. D. Fitzgerald

TABLE 1. Abortion Data for Mares Dosed with Eastern Tent Caterpillars and Isolation of Bacteria from Aborted Fetuses

Dose of Eastern Tent Caterpillars	Time of Abortion (hr)	Bacteria Isolated from Aborted Fetuses
50 g/day × 10 days		
Mare 220	62	*Enterobacter sakazaki*
Mare 78	166	*Serratia marcescens,* *Enterococcus* spp
Mare 398	142	*Enterobacter cloacae*
Mare 390	95	*E. cloacae*
Mare 395	69	*E. cloacae*
Mare 393	350	*S. marcescens*
100 g/day × 10 days		
Mare 647	32	*S. marcescens*
Mare 668	32	*S. marcescens*
Mare 638	46	*S. marcescens*
Mare 305	48	*S. marcescens*
Mare 630	84	*S. marcescens*
Mare 10	120	*S. marcescens*
100 g/day × 10 days*		
Mare 637	280	*Streptococcus* spp
Mare 619	326	*Actinobacillus* spp
Mare 172	567	*Streptococcus* spp
Mare 628	Did not abort[†]	
Mare 351	Did not abort[†]	
Mare BUC	Did not abort[†]	

*Irradiated caterpillars.

[†]As of submission of this report for publication.

deionized water or saline and ground in a blender for 20 to 30 minutes before administration. Mares were examined daily by ultrasound after treatments were initiated. Aborted fetuses were transported to the laboratory for examination and isolation of bacteria by routine aerobic culture.

Daily LFL abortion data from the 2001 MRLS outbreak in central Kentucky submitted to the Livestock Disease Diagnostic Center (LDDC) and reported to the USDA Animal and Plant Health Inspection Service Veterinary Services, Frankfort, Kentucky were also evaluated.

Regression Analysis

The data points from the two studies in which ETCs were fed to pregnant mares were first plotted as a percentage of total abortions against time, and best-fit logarithmic regressions were calculated. The x-intercepts (apparent "lag times") were calculated by setting the value of y equal to zero in the equation.

source in northern Michigan because the season had passed in Kentucky. In 2003, the effects of irradiation on the abortigenic efficacy of ETCs were evaluated in groups of six mares in late pregnancy by administration of 100 g of either irradiated or nonirradiated ETCs via nasogastric tube for 10 days.[17] Doses of caterpillars in each study were determined from previous work conducted in 2002.[15] Irradiation was used as a means to destroy any bacteria or virus that might be carried by the caterpillars. The caterpillars were mixed with

Accelerated Failure Time Model

The data points were also fitted using the AFT survival model because this model is one of regression that allows for the prediction of the time at which events occur based on independent variables. In this case, the time at which abortions occur was predicted based on the dose of ETCs administered. The AFT model can also take censored data (an event that had not occurred by the time the experiment had ended) into account. In these studies, mares that had been dosed with ETCs but

had not aborted by the end of the data collection period were considered censored observations. A log-normal AFT model has been used to fit the ETC data. That is, if T represents the time at which abortion occurs, log T is assumed to have a normal distribution, and thus T has a log-normal distribution. The model also assumes that the error term is normally distributed. The model used here assumes that the probability of an abortion event remains close to zero for a period after the first administration, which is dependent on the dose administered. This model takes the form:

$$T_i = \exp(\beta_0 + \beta_1 x_{i1} + \ldots + \beta_k x_{ik} + \sigma \varepsilon_i)$$

where T is time, x is a variable that might affect abortion time (i.e., dose of ETCs), β_0, β_1, etc are coefficients that estimate the effect of the x variables (i.e., abortion time), ε is an error term that is assumed to be normally distributed, and σ is a scale parameter for the error term.

A likelihood ratio test of the fitted AFT model versus a null model (with no explanatory variables) yields a chi-square statistic of 27.9 with 2 degrees of freedom. This statistic is significant at the $P \leq .0001$ level, which indicates that the model with parameters ETC dose and irradiation is a significant improvement over the null model for predicting time until abortion. The individual chi-square tests for these effects are also highly significant ($P \leq .01$), which indicates both variables are important to the model. The model differs from the Cox regression model in that it assumes an underlying distribution for the data (log-normal, in this case), whereas the Cox regression model does not require such an assumption.

Based on the AFT analysis, time courses of abortion responses were projected for various doses ranging from 5 to 500 g of ETC/day. The AFT model and this family of dose response curves allow dose and time response in-

terpretation of any ETC-related sequence of abortions in terms of the dosage unit used in these experiments, which have been defined as "intubated ETC equivalents."

A constraint in this model is that none of the ETC dosings continued beyond 10 days, whereas AFT analysis assumes that dosing or exposure to the caterpillars is continuous. Because of this, the mathematical fit of the AFT model to the data is presumably more accurate at the higher doses rather than at the doses used with irradiated ETCs, which exhibited an apparently much lower abortigenic activity. A further constraint is that the duration of field exposure to dispersing ETCs at any given location is rarely longer than 20 days.

■ RESULTS
Linear Regression Analysis

In the first experimental reproduction of the LFL syndrome (2002), all mares given 50 g of ETCs/day for 10 days aborted rapidly (within 14 days). The first abortions occurred within 72 hours (Table 1). In the 2003 study, mares receiving 100 g of nonirradiated ETCs/day began aborting within 32 hours and all had aborted by Day 5 (Table 1). In contrast, only three of the six mares receiving irradiated ETCs had aborted by the time of this writing (70 days after the initial dosing), and these mares did not begin aborting until Day 12, 2 days after the last dose of ETCs (Table 1). Furthermore, the most recent abortion from the group given irradiated ETCs was observed 24 days after the initial exposure to ETC, which was 14 days after the last administration. Regressions for each dose/abortion time data set were then calculated (Figure 1).

The abortion curves for each of these studies were similar in shape and were apparently related (Figure 1). The curve for 100 g/day was shifted to the left compared with that for 50 g/day, suggesting a dose-response effect. Clas-
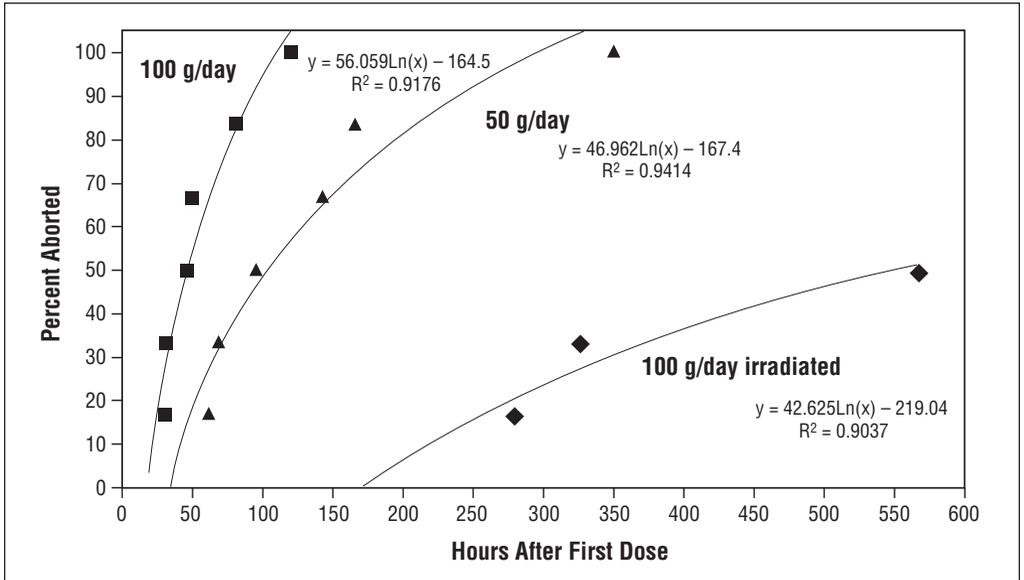
M. Sebastian, M. G. Gantz, T. Tobin, J. D. Harkins, J. M. Bosken, C. Hughes,
L. R. Harrison, W. V. Bernard, D. L. Richter, and T. D. Fitzgerald

Figure 1. *Time course of abortions following intubation of mares with 50 and 100 (nonirradiated or irradiated) g of Eastern tent caterpillars (ETCs)/day for 10 days. The solid lines are the best-fit regressions for the data points. The calculated x-axis inteceps (apparent lag times) are 20 (100 g), 37 (50 g), and 193 hours (100 g irradiated) after the first dose of ETCs.*

sic dose-response curves show parallel slopes, but the slopes of these curves were markedly different, with the steeper slope of the curve for 100 g/day, presumably relating to the higher dose of ETCs administered. Interestingly, each fitted curve appeared to intersect the x-axis at well-defined time points (approximately 20, 37, and 193 hours, for 100, 50, and 100 irradiated g/day, respectively), which invited further analysis. These x-axis intercepts (lag times) also appeared to be dose related.

Survival Analysis

Evaluation of the data using the Cox regression analysis (PHREG procedure; SAS version 8, SAS Institute), yielded an unsatisfactory data fit (data not shown). However, application of the data points to AFT analysis (LIFEREG procedure; SAS version 8, SAS Institute) produced a very satisfactory fit. Cumulative distribution functions for each group, or cumulative abortion curves, based on the model are presented in Figure 2. The AFT analysis assumes that the data points are log-normally distributed and also reflects the fact that the probability of an abortion remains close to zero for a dose-dependent period of time after dosing begins (the apparent lag time). The AFT model was then used to estimate the time after dosing that abortion occurred. Using the data presented above, AFT regression yielded the following model:

$$T_i : \exp(5.477 - 0.017\ \text{dose}_i + 2.182\ \text{irradiated}_i + 0.526\ \varepsilon_i)$$

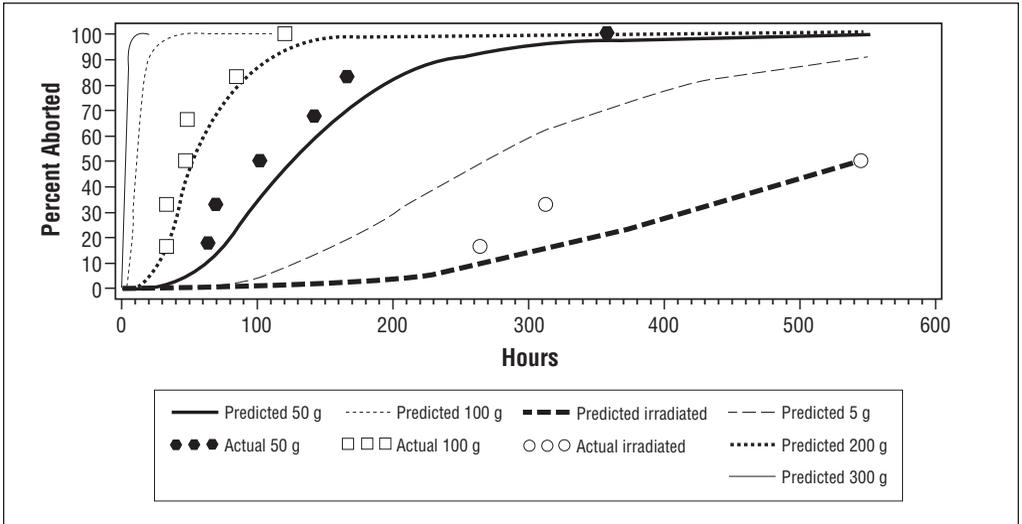|			
Standard error:	0.480	0.006	0.353	0.101
P:	<.0001	.0052	<.0001	

Figure 2. *Actual abortion rates following dosing with Eastern tent caterpillars (ETCs) at 50 and 100 (nonirradiated and irradiated) g/day for 10 days and predicted abortion rates by the accelerated failure time model for doses of 5, 50, 100 (nonirradiated and irradiated), 200, and 300 g ETCs/day. The estimated lag times for the actual ETC doses administered are 10 (100 g), 20 (50 g), and 80 (100 g irradiated) hours.*

where dose$_i$ is the dose of caterpillars for individual i, and irradiated$_i$ equals 1 if individual i was fed irradiated and 0 if not. The coefficients indicate that the time until abortion decreased with increasing dose and increased if the ETCs were irradiated. All coefficients were highly significant ($P \leq .01$) in the model.

The estimated lag times were 10, 20, and 80 hours for 100, 50, and 100 irradiated g/day, respectively (Table 2). Because of the excellent fit of the data to the AFT model, cumulative distribution curves based on the model were plotted for several doses of caterpillars (Figure 2).

Daily and cumulative time courses of aborted foals submitted to the LDDC from March 1 through May 12, 2001 are shown in Figure 1. In March and April (before administration of ETCs was initiated), the abortion rate was approximately nine per day. The increased slope and divergence from the prediction line began around April 30 and represents abortions due to MRLS, which averaged around 42

abortions per day and peaked at 66 abortions per day (May 4).

The data can also be used to estimate the time of first exposure to the ETCs. Assuming that any abortion would have occurred the day before the accession is recorded in the LDDC, and that the first abortions were due to low-level ETC exposure producing a lag time of about 50 hours for the group given 5 g ETCs/day (Table 2), the first exposure to abortigenic ETCs in the 2001 outbreak was estimated to have occurred on April 26 or 27. Inspection of the cumulative abortion curve also shows a further increase in the rate of abortions on May 4 (Figure 3).

To estimate the level of exposure to ETCs necessary to produce the abortion rates observed during the 2001 MRLS outbreak, the cumulative time course of MRLS–related abortions (Figure 3) was converted to a percentage of abortions and compared with the AFT–calculated abortion rates following exposure to 5

M. Sebastian, M. G. Gantz, T. Tobin, J. D. Harkins, J. M. Bosken, C. Hughes,
L. R. Harrison, W. V. Bernard, D. L. Richter, and T. D. Fitzgerald

TABLE 2. Estimated Time Until 0%, 50%, and 100% of Abortions Occur in Pregnant Mares Following Oral Administration of Doses (g/day) of Eastern Tent Caterpillars for 10 Consecutive Days

| Predicted % to Abort | Estimated Time to Abortion (hr) at Given Dose of Eastern Tent Caterpillars (g) | | | | | | |
	100 (irradiated)	1	5	50	100	200	300
0	80	55	50	20	10	2	0.3
50	470	285	266	124	53	9.8	1.8
100	4,900	3,000	2,800	1,300	550	100	19

and 30 g/day (Figure 4). The first part of the cumulative curve coincides with the curve for 5 g/day; however, the last portion of the cumulative curve overlays the curve for 30 g/day when it is shifted to the right by 80 hours.

The calculations assume that no abortions occurred due to caterpillar ingestion prior to April 25 and that 100% of the mares exposed to caterpillars eventually aborted. It also assumes that approximately 50 abortions were yet to occur between May 12 and May 19. The analysis suggests that exposure to ETCs during the MRLS outbreak of 2001 likely started at the equivalent of approximately 5 g of intubated ETCs/day in late April and then increased to around 30 g/day around May 4, the peak of the outbreak.

The model was also used to explore the relationships between dose of ETCs and daily abortion rate. Daily abortion rates following various ETC doses based on the AFT model are shown in Figure 5. The graph assumes there are 600 mares in each treated group and that all treated mares will eventually abort. The analysis demonstrates that exposure to 100 g of ETCs/day rapidly causes abortions that peak 3 to 4 days after exposure, and essentially all of the exposed mares will abort by Day 10. At a dose of 30 g/day, abortions peak approximately 6 days after exposure and are complete within approximately 18 days. If the abortigenic dose of caterpillars is reduced to an amount equivalent to that present in the irradiated dose (100 g/day), the first abortions do not begin to appear until about Day 8, and the abortions will not peak until Day 20.

A proposed schematic of the AFT model of MRLS is presented in Figure 6. In this model, continuous exposure to ETCs commencing at Time 0 is followed by a dose-dependent lag time, after which abortions occur. The duration of the lag time, the initial maximal rate at which the abortions occur, and the time until 100% of the exposed mares abort are all directly related to the dose of ETCs and presumably to the rate of ETC exposure.

The bacteria isolated from the aborted fetuses were *Serratia marcescens, Enterococcus* spp, and *Enterobacter* spp for the mares given 50 g of ETCs/day, *S. marcescens* for those given 100 g/day, and *Streptococcus* and *Actinobacillus* spp in the irradiated study (Table 1).

■ DISCUSSION

The data sets were initially approached as clinical data, and the time course of each sequence of abortions as plotted. Inspection of the data and the apparent fit to the linear regression model drew attention to the fact that the curves were members of a family of curves that appeared to commence at a time significantly different from zero. Additionally, the differences from Time 0 and the maximal slope
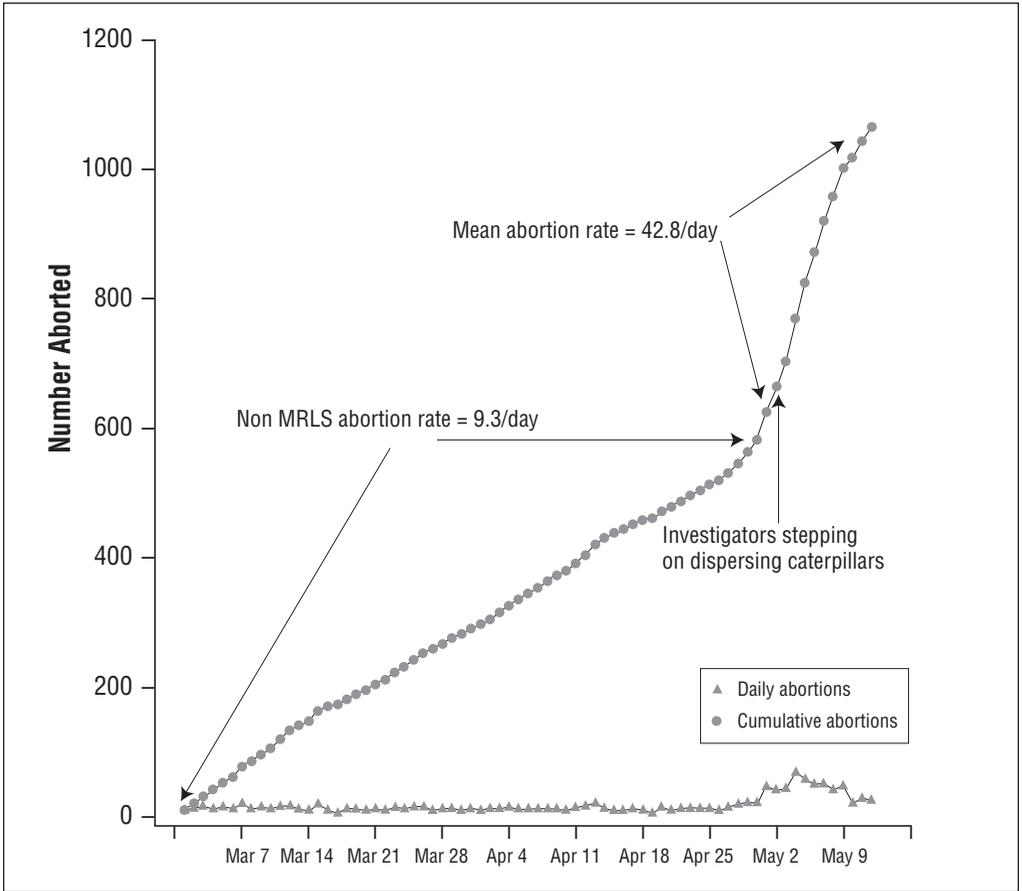
Figure 3. *Daily and cumulative number of abortions submitted to the Livestock Disease Diagnostic Center in Lexington, Kentucky between March 1 and May 12, 2001. The increased slope of the cumulative abortion line (May 1 to May 12) is due to MRLS onset, peaking at about 66 MRLS abortions per day on May 4. From these curves, the first MRLS abortions started on approximately April 30, 2001.*

of the curves appeared to vary with dose. These observations, therefore, led to the more detailed analysis. Because the AFT survival analysis is appropriate for constructing models that relate an event to the time at which it occurs, it was the model selected to fit these data points, and all coefficients were statistically significant. The model was then used to estimate dose × abortion time curves for a series of doses ranging from 5 to 500 g/day. The very good

fit of the ETC dose × abortion time data by the AFT model provides a solid statistical basis for integration and interpretation of experimental and field MRLS data. These results and interpretations are best summarized in the schematic AFT model of ETC–induced abortions in mares presented in Figure 6. Early in the exposure period, the lag time represents the period during which the likelihood of an abortion occurring remains close to zero. Following the lag
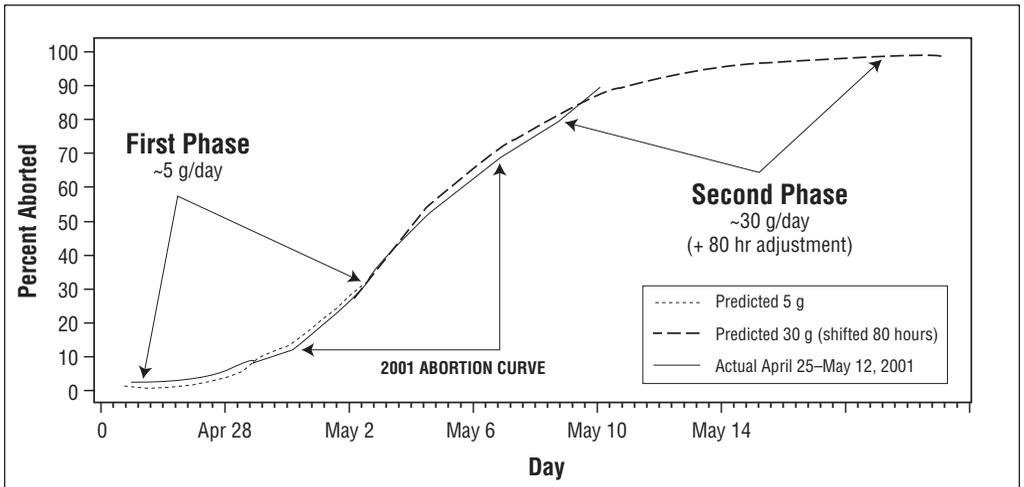
M. Sebastian, M. G. Gantz, T. Tobin, J. D. Harkins, J. M. Bosken, C. Hughes,
L. R. Harrison, W. V. Bernard, D. L. Richter, and T. D. Fitzgerald

Figure 4. *The cumulative actual percentage of abortions that occurred between April 25 and May 12, 2001 (taken from data shown in Figure 3) plotted with the predicted curves for the cumulative percentage of abortions that would have occurred following doses of 5 and 30 g of Eastern tent caterpillars per day. The cumulative abortion curve for 30 g/day is shifted to the right by 80 hours, showing that the last 60% of the 2001 abortions essentially overlapped that dose. The figure implies that the dose of caterpillars ingested by the mares in 2001 likely started at around 5 g/day in late April and was related to abortions in approximately 30% of the exposed mares. In the second phase of abortions in early May, the apparent dose increased to around 30 g/day, with approximately 60% of the exposed mares aborting around that time. The entire sequence of events, from initial exposure to abortion of 90% of the exposed mares, took no more than 3 weeks.*

time, the rate at which abortions occur accelerates to the peak abortion rate. The abortions then proceed until either all of the exposed mares abort or until some time (currently unknown) after which exposure to ETCs has ceased. In studies with small numbers of horses (i.e., six), as presented in Figure 2, the fine detail of the abortion rate transformation from zero in the lag-time phase to the maximal abortion rate is inevitably lost.

This analysis makes clear that the rate at which abortions occur (rather than the absolute number of abortions) determines the relative abortigenic efficacy of any ETC treatment. The AFT model predicts that ETC doses between 5 and 500 g/day will, if maintained, sooner or later abort 100% of the exposed mares.

The decreased rate of abortions for irradiated ETCs suggests that the efficacy of the ETC abortion–inducing factor was substantially reduced by irradiation or something associated with this treatment, since the first abortion did not occur until 12 days after the first ETC administration. The abortions then proceeded slowly and, according to AFT analysis, the time to the last abortion (100%) will be about 206 days, assuming continuous dosing. Based on the AFT model, the apparent efficacy of the abortigenic factor in irradiated ETCs is less than 1 g of untreated ETCs/day, for an apparent loss of >99% of the ETC–related abortigenic activity.

Loss of such a substantial fraction of the abortigenic activity in the irradiated ETCs is surprising but not inconsistent with recent experimental results. Earlier research,[18] which was later confirmed by Webb and McDowell,[19] has shown that the abortigenic factor is asso-
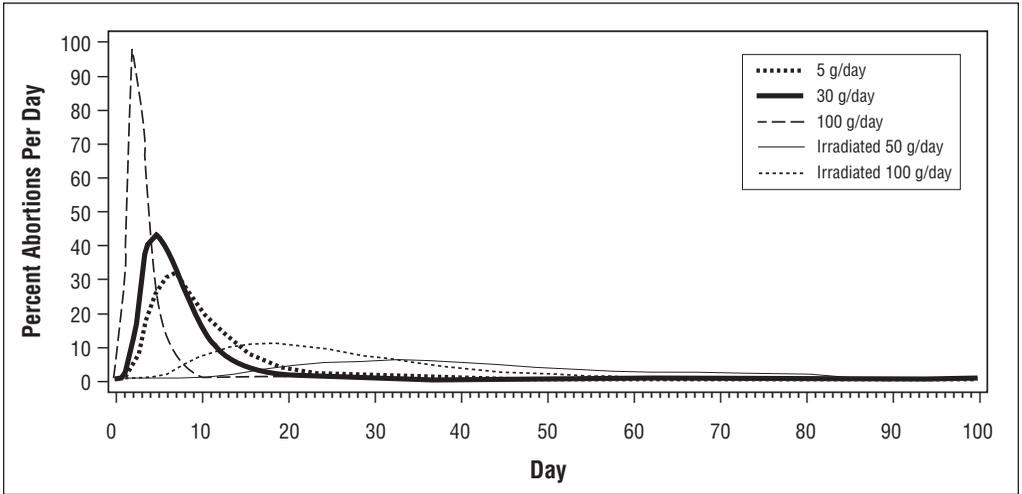
Figure 5. *Effect of dose on daily abortion rates as a function of daily exposure to Eastern tent caterpillars (ETCs) for 600 mares. At 100 g of ETCs/day, the abortions occur rapidly, peaking approximately 3 to 4 days after exposure, and virtually all susceptible mares exposed will abort by Day 10. At 30 g/day, the abortions peak about 6 days after exposure and are largely complete within approximately 18 days. If the abortigenic dose of caterpillars is reduced to an amount equivalent to that present in the irradiated caterpillars, the first abortions do not begin to appear until about Day 8, and the abortions will not peak until Day 20. The model assumes that there are 600 mares in each group and that all mares dosed with ETC will eventually abort.*

ciated with the cuticle of ETCs. While fractionating the ETC integument in a series of deductive approaches to identifying the ETC abortigenic factor, Webb and McDowell readily lost a substantial fraction of the ETC–related abortigenic activity, which may suggest significant fragility of the abortigenic factor or factors closely associated with it.

The mathematical relationships described by the AFT model explain many aspects of the epidemiology and clinical presentations of MRLS. If the exposure to ETCs is high, all susceptible exposed mares are likely to abort within in a relatively short period. In this regard, transformation of the cumulative fetal loss curve for 30 g/day (Figure 2) into a daily "rate of abortion" curve (Figure 5) closely follows the general shape of the accessions curve to the LDDC around May 6, 2001. Of critical importance during the 2001 investigation of

MRLS, when the dose of ETCs is high and the abortions occur rapidly, there will be an obvious temporal relationship between the presence of caterpillars and the abortions, as occurred during 2001. In fact, it was the close spatial and temporal relationships between the presence of the ETCs and the appearance of MRLS,[b] as well as the lack of any other convincing hypothesis, that led to the early conclusion that ETCs were most likely associated with MRLS.

Significant objections were raised to the hypothesis that the ETCs were involved in the pathogenesis of MRLS. Beyond entomologic and common experience that ETCs were "known to be harmless," the question was raised as to why the syndrome had never been

---

[b]Henning J: Personal communication, Department of Agronomy, University of Kentucky, Lexington, KY, 2001.
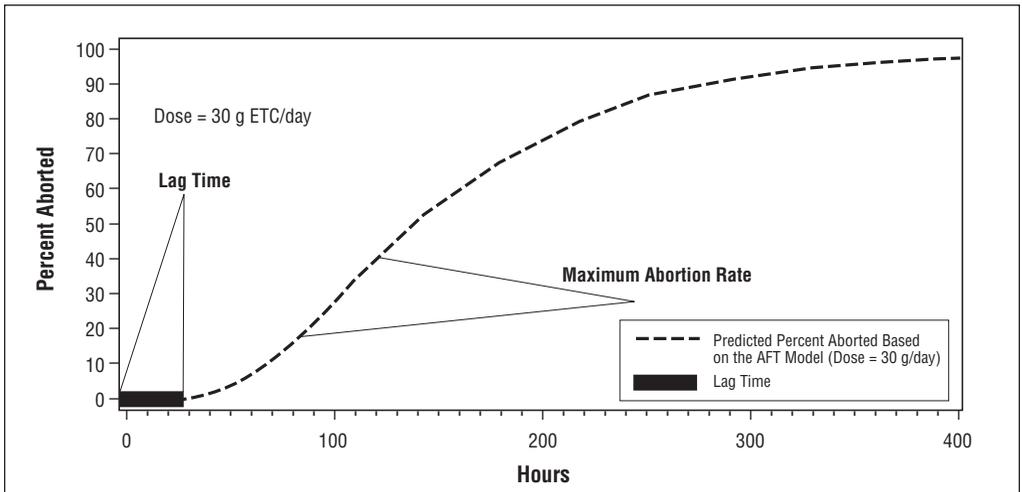
Figure 6. *Proposed schematic accelerated failure time model for MRLS. Continuing exposure to Eastern tent caterpillars (ETCs) commencing at Time 0 is followed by a dose-dependent lag time, indicated by the solid horizontal bar, after which the abortions commence. The duration of the lag time, the initial maximal rate at which the abortions occur, and the time to abort 100% of the exposed mares are all directly related to the dose of ETCs and, presumably to the rate of ETC exposure.*

identified previously in Kentucky, since ETC infestations are not uncommon. However, the analysis presented here shows that if the exposure to caterpillars is less intense, the lag time is longer and the abortions will tend to be spread out over time and occur out of phase with ETC exposure. For example, data from the irradiation experiment indicates that at low doses the first abortions may not occur until after ETC exposure has ceased, and the vast majority of the statistically predicted abortions may occur at a time distant from the presence of ETC.

Another factor that probably limits the abortigenic efficacy of low doses of ETCs is the relatively short time mares are field exposed to the caterpillars. Field exposure to ETCs is not continuous but is limited by the relatively brief ETC dispersal period as they wander about during the day for not more than 2 weeks in total as the sixth and final instars search for food and suitable pupation sites. Exposure to

high concentrations of ETCs during this brief dispersion time will rapidly abort most, if not all, of the exposed mares, as indicated by the experimental data and the clinical picture in central Kentucky during the 2001 outbreak. In the intense exposure scenario, the full abortifacient potential of the high dosage of ETCs is inevitably expressed.

Conversely, while the AFT model predicts that continuous exposure to low doses of ETCs will eventually abort all exposed susceptible mares, this is not likely to happen in the field, where exposure is transient. Once ETC exposure stops, the abortion process eventually ceases, and the full abortion potential of a low dose is never achieved. As such, with lower doses of ETCs, the lag time is longer, and the actual fraction of possible abortions likely to occur is smaller.

In such low-exposure circumstances, the ETCs are less likely to be associated with the abortion events, which will be more diffuse

and will tend to meld into the normal background rate of fetal loss, and will draw little clinical attention. In this regard, careful review of clinical records from the previous year by equine practitioners in central Kentucky has retrospectively identified cases of MRLS occurring during the 2000 ETC season, before MRLS had ever been described.[c]

In retrospect, it is easy to see how previous episodes of what is now recognized as MRLS in central Kentucky during the 1980 and 1981 breeding seasons were never linked to the caterpillars. Ultrasound technology was not available in the 1980s, so EFL was not recognized until spring-bred mares were checked for pregnancy in the fall, following 1980s management practices. As such, the critical physical association of ETCs with the abortion events was missed.

In contrast, the critical factor in the identification of MRLS with ETCs in 2001 was the high concentration of pregnancies closely monitored first via ultrasound by highly skilled practitioners, and shortly thereafter by skilled pathologists and veterinary scientists. EFL was recognized within hours of its first appearance; infectious causes were very rapidly ruled out; and the syndrome was defined and described within weeks. The caterpillars were rapidly pinpointed as the likely cause, and an intensive search for a caterpillar-associated toxin or factor was underway by Week 3.

During the 2003 season, there have been at least two other substantial caterpillar infestations distant from Kentucky, one in New York involving ETCs, and one in Washington involving Western tent caterpillars. However, these areas do not have a sufficient number of pregnant broodmares nor have they been monitored adequately to establish whether MRLS or another caterpillar-related syndrome was present. To date, MRLS has not been reported

outside of Kentucky and its adjacent states.

The direct relationship between ETC exposure and the abortion rate suggested that it should be possible to estimate the rate of ETC exposure occurring during the 2001 MRLS outbreak. In performing this analysis, it should be understood that not all horses in central Kentucky were exposed to ETCs. As such, it is not possible to estimate the overall exposure rates to ETCs throughout the area, but the levels of ETC exposure needed to produce the actual abortion rates observed as accessions to the LDDC during the 2001 MRLS outbreak can be estimated.

To perform this analysis, the daily abortion accessions rates to the LDDC were transformed into a cumulative abortion curve, showing that they follow the same general patterns as produced by the AFT analysis (Figure 4). Based on this analysis, it appears that the initial rate of ETC exposure, as set forth by the first one-third of field abortions in 2001, was equivalent to a dose of about 5 g/day, which is equivalent to approximately 15 ETCs/day. Thereafter, the abortion rate accelerated, consistent with a rate equivalent to approximately 30 g/day for the last two-thirds of the field abortions.

The potential intensity of exposure to ETCs during the 2001 MRLS outbreak is illustrated by the number of ETCs on a water bucket at a central Kentucky horse farm (Figure 7). With regard to the temporal relationship between the presence of the caterpillars and the onset of LFL during the 2001 MRLS outbreak, at approximately 2 PM on Wednesday, May 2, an epidemiologist from the University of Kentucky visited a Kentucky farm in response to an outbreak of LFL. He stepped out of his car in the office driveway onto dispersing ETCs, unavoidably stepping on huge numbers of caterpillars underfoot as he proceeded to the farm office. Later that afternoon, he and an as-

Figure 7. *Water bucket on a Kentucky horse farm demonstrating the intensity of exposure of horses to Eastern tent caterpillars during spring of 2001. (Photo courtesy of Henry Q. Murphy).*

sociate were called to another thoroughbred farm for assistance with an epidemic of EFL.[d] The week before the 2001 Kentucky Derby, unparalleled numbers of ETCs were dispersing in central Kentucky, and EFL and LFL were approaching their peaks (Figure 3).

Finally, this analysis must be put into perspective. The MRLS initially consisted of four distinct syndromes, EFL, LFL, pericarditis, and unilateral endophthalmitis. Nevertheless, two other disorders also occurred in association with exposure to ETCs that have not been included in the official definition of MRLS but must be incorporated in any analysis of the basic mechanisms of the syndrome. These include the birth of a significant number of late-term weak foals[20] with up to 50% mortality, and the more recently identified small number of cases of *Actinobacillus* encephalitis also associated with the 2001 ETC season.[21] As such, MRLS encompasses at least six different clinical presentations, and this analysis only ad-

dresses LFL syndrome in depth and is based primarily on acute high-dose exposures to ETCs.

An unusual aspect of MRLS is the very wide spectrum of bacterial species isolated from aborted foals.[22] The close fit of the experimental data to the AFT model may suggest that the role of different bacterial species in the abortion events is not a critical determinant of the time to abortion, or of the fundamental mechanism that drives this syndrome.

The conclusions drawn from this analysis are valid for LFL and throw much light on the epidemiology and clinical presentation of the 2001 MRLS outbreak. The analysis also carries some clear messages for further work. The first thing to consider for any attempts to quantitatively relate experimental ETC exposure to abortion responses is that the database must include abortions of all exposed mares. This is because it is difficult to estimate the rates of abortion if only a small fraction of the exposed mares have aborted and, as this analysis shows, it is the rate at which the abortions occur that relates directly to the dose of ETC abortigenic activity. It is acknowledged that there is a possibility of different results if a larger population of animals (and additional doses of ETCs) were included in the AFT analysis; however, the data evaluated by the model were highly suggestive of the conclusions drawn.

The second factor, and the one that is not addressed in this analysis, is the apparent ability of horses and late-term fetuses in utero to resist the mechanism of ETC–induced abortions and the other syndromes associated with MRLS. The birth of a large number of weak foals with high mortality during the 2001 ETC season is

---

[d]Powell D: Personal communication, University of Kentucky, Gluck Equine Research Center, Lexington, KY, 2001.

consistent with an MRLS–related subclinical disease that failed to produce "dead foal" cases of LFL. These cases and the small number of pericarditis and uveitis cases illustrate a substantial resistance to the pathogenic mechanism of MRLS in late-term fetuses and in horses in general, which needs to be taken into account in any model of the pathogenesis of MRLS.

The quantitative aspects of the interactions between ETCs and late-term pregnant mares set forth in this communication are unusual and suggest a rate-limiting, quantal, irreversible step in the pathophysiology of the MRLS.

Reviewing the unique spectrum of clinical syndromes that comprise MRLS, the authors have suggested that the fundamental underlying pathophysiologic mechanism of MRLS is the ability of barbed ETC setal fragments to penetrate moving tissues, thereby mechanically facilitating entry of miscellaneous bacteria ("bacterial hitchhikers") carried on (or in) such septic penetrating setal fragments.

Following oral exposure to ETCs, these septic setal fragments penetrate the gastrointestinal tract and intestinal blood vessels, through which septic materials distribute to distant tissues following cardiac output. The redistributing septic materials may be bacteria, bacterial emboli, or small, septic setal fragments. In most tissues, such septic materials are readily handled by the immune system. In tissues containing a significant volume of extracellular fluid, bacterial proliferation may be less well controlled. Entry of septic materials into the fetal fluids results in rapid bacterial proliferation, followed by the EFL and LFL syndromes. Distribution to one eye of a quantum of septic materials yields the low incidence of unilateral uveitis that is virtually unique to MRLS. The pericarditis lesions reflect the central role of the heart in the circulatory system and its unusually high exposure to circulating septic materials.

This mechanism is quantal in nature, and the effect on the early fetus, once penetration of the fetal membranes has occurred, is essentially irreversible. The hypothesis, which has previously been presented,[18,23,24] explains the unusual quantitative aspects of MRLS presented here, as well as the unusually broad spectrum of bacterial pathogens involved in MRLS. It is also consistent with the historical ability of setal fragments to penetrate moving tissues and the more recent identification of large numbers of setal fragments encased in microgranulomas reported in the intestinal tracts of ETC treated pigs.[25] This hypothesis has been set forth in detail, and is the basis of a forthcoming publication.[26]

■ REFERENCES

1. Harrison LR: Kentucky abortion storm and related conditions. *Proc US Anim Health Assoc* 105:227–229, 2001.
2. Dwyer RM, Garber LP, Traub-Dargatz JL, et al: Case-control study of factors associated with excessive proportions of early fetal losses associated with mare reproductive loss syndrome in central Kentucky during 2001. *JAVMA* 222:613–619, 2003.
3. Williams NM, Bolin DC, Donahue JM, et al: Gross and histopathological correlates of mare reproductive loss syndrome, in Powell DG, Troppmann A, Tobin T (eds): *Workshop on Mare Reproductive Loss Syndrome.* Lexington, KY, University of Kentucky College of Agriculture, 2002, pp 24–27.
4. Slovis NM: Clinical observations of the pericarditis syndrome, in Powell DG, Troppmann A, Tobin T (eds): *Workshop on Mare Reproductive Loss Syndrome, 2002.* Lexington, KY, University of Kentucky College of Agriculture, 2002, pp 18–20.
5. Latimer C: Endophthalmitis: A syndrome associated with mare reproductive loss syndrome?, in Powell DG, Troppmann A, Tobin T (eds): *Workshop on Mare Reproductive Loss Syndrome, 2002.* Lexington, KY, University of Kentucky College of Agriculture, 2002, pp 17–18.
6. Riddle WT: Clinical observations associated with early fetal loss in MRLS during the 2001 and 2002 breeding seasons, in Powell DG, Troppmann A, Tobin T (eds): *Workshop on Mare Reproductive Loss Syndrome.* Lexington, KY, University of Kentucky College of Agriculture, 2002, pp 12–14.
7. Schultz CL, Bush LP: The potential role of ergot alka-

loids in mare reproductive loss syndrome, in Powell DG, Troppmann A, Tobin T (eds): *Workshop on Mare Reproductive Loss Syndrome.* Lexington, KY, University of Kentucky College of Agriculture, 2002, pp 60–63.

8. McDowell K, Allman R, Henning J: Phytoestrogens and estrogenic activity in white clover samples from no-loss and high-loss fields during mare reproductive loss syndrome, in Powell DG, Troppmann A, Tobin T, (eds): *Workshop on Mare Reproductive Loss Syndrome.* Lexington, KY, University of Kentucky College of Agriculture, 2002, pp 63–65.

9. Newman K: Review of mycotoxins as a possible cause of mare reproductive loss syndrome, in Powell DG, Troppmann A, Tobin T (eds): *Workshop on Mare Reproductive Loss Syndrome.* Lexington, KY, University of Kentucky College of Agriculture, 2002, pp 66–68.

10. Harkins JD, Dirikolu L, Sebastian M, et al: Cherry trees, plant cyanogens, caterpillars, and the mare reproductive loss syndrome (MRLS): Toxicological evaluation of a working hypothesis, in Powell DG, Troppmann A, Tobin T (eds): *Workshop on Mare Reproductive Loss Syndrome.* Lexington, KY, University of Kentucky College of Agriculture, 2002, pp 68–74.

11. Fitzgerald TD, Jeffers PM, Mantella D: Depletion of host-derived cyanide in the gut of the eastern tent caterpillar, *Malacosoma americanum. J Chem Ecol* 28:257–268, 2002.

12. Cohen ND, Carey VJ, Donahue JG, et al: Descriptive epidemiology of late-term abortions associated with the mare reproductive loss syndrome in central Kentucky. *J Vet Diagn Invest* 15:295–297, 2003.

13. Dwyer RM: Epidemiological correlates of the 2001 and 2002 episodes of mare reproductive loss syndrome, in Powell DG, Troppmann A, Tobin T (eds): *Workshop on Mare Reproductive Loss Syndrome.* Lexington, KY, University of Kentucky College of Agriculture, 2002, pp 34–36.

14. Webb B, Barney WE, Dablman DL, et al: Induction of mare reproductive loss syndrome by directed exposure of susceptible mares to Eastern tent caterpillar larvae and frass, in Powell DG, Troppmann A, Tobin T (eds): *Workshop on Mare Reproductive Loss Syndrome.* Lexington, KY, University of Kentucky College of Agriculture, 2002, pp 78–79.

15. Bernard B, Webb B, LeBlanc M: Gastric administration of Eastern tent caterpillars causes early fetal loss in pregnant mares, in Powell DG, Troppmann A, Tobin T (eds): *Workshop on Mare Reproductive Loss Syndrome.* Lexington, KY, University of Kentucky College of Agriculture, 2002, pp 79–80.

16. Sebastian M, Willaims D, Harrison LR, et al: Experimentally induced mare reproductive loss syndrome late fetal losses with Eastern tent caterpillars, in Powell DG, Troppmann A, Tobin T (eds): *Workshop on Mare Reproductive Loss Syndrome.* Lexington, KY, University of Kentucky Department of Agriculture, 2003, pp 80–81.

17. Cox N, Miller H: Mare Reproductive Loss Syndrome, *Researchers Get Closer to Solving Caterpillar Mystery.* Available at http://www.uky.edu/Agriculture/VetScience/mrls/062603closertosolving.html, June 26, 2003.

18. Tobin T: Mare reproductive loss syndrome and associated syndromes: Toxicological hypothesis: Summary, in Powell DG, Troppmann A, Tobin T (eds): *Workshop on Mare Reproductive Loss Syndrome.* Lexington, KY, University of Kentucky College of Agriculture, 2003, pp 75–76.

19. McDowell K, Webb B: *Deductive Investigation of the Causes of Mare Reproductive Loss Syndrome: Experiment 4.* Available at http://www.uky.edu/Agriculture/VetScience/mrls/DeductiveInvestigation060603.html; June 6, 2003.

20. Byars TD, Seahorn TL: Clinical observations of mare reproductive loss syndrome in critical care mares and foals, in Powell DG, Troppmann A, Tobin T (eds): *Workshop on Mare Reproductive Loss Syndrome.* Lexington, KY, University of Kentucky College of Agriculture, 2003, pp 15–16.

21. Sebastian M, Giles RC, Donahue J, et al: Encephalitis due to *Actinobacillus* species in three adult horses [Abstract]. *Vet Pathol* 39:630, 2002.

22. Donahue JM, Sells S, Giles RC, et al: Bacteria associated with mare reproductive loss syndrome, in Powell DG, Troppmann A, Tobin T (eds):*Workshop on Mare Reproductive Loss Syndrome.* Lexington, KY, University of Kentucky College of Agriculture, 2003, pp 27–29.

23. Tobin T: Septic Penetrating Setal Emboli. *Bain Fallon Lecture Series* The Gold Coast, Australia: July 26, 2002.

24. Tobin T: Septic penetrating setae/septic emboli/septic penetrating setal emboli (SPS/SE/SPSE): A hypothesis to explain the pathogenesis of the mare reproductive loss syndrome. *Copyright registered at Library of Congress, June 17, 2003,* No. TXU1111484, 2003.

25. McDowell K, Williams NM, Donahue JM, et al: *Eastern Tent Caterpillars Cause Abortions in Pregnant Pigs.* Available at http://www.uky.edu/Agriculture/VetScience/mrls/090503ETCabortpigs html; September 5, 2003.

26. Tobin T, Harkins JD, VanMeter PW, Fuller T: Clinical evaluation and a proposed mechanism of the mare reproductive loss syndrome: Septic penetrating setal emboli. *Vet Ther*, submitted for publication, 2003.