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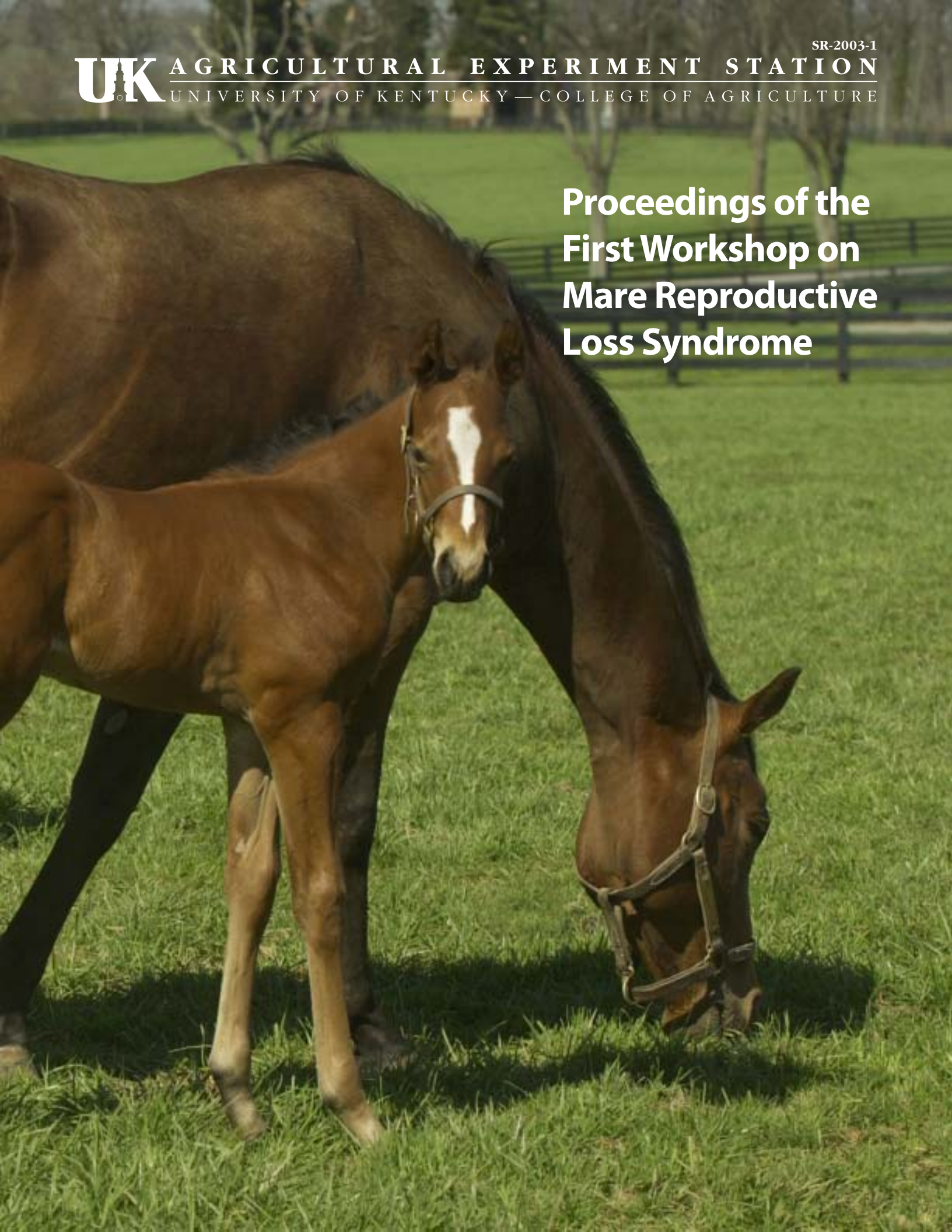

Proceedings of the First Workshop on Mare Reproductive Loss Syndrome

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edited by

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Maxwell H. Gluck Equine Research Center • Lexington, Kentucky 40546

First Workshop on Mare Reproductive Loss Syndrome, 2002

hosted by
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Contents

Preface

The dramatic emergence of Mare Reproductive Loss Syndrome (MRLS) among the horse population of Kentucky in April of 2001 and 2002 has had a tremendous impact on the equine industry. The consequences as a result of the significant reduction in the foal population will be felt for several years to come at the state and national level, even extending around the world.

The response to this acute crisis produced an unprecedented collaborative effort to identify the cause. This effort involved scientists, practicing veterinarians, individuals, and organizations representing every facet of the equine industry, both nationally and internationally.

Although the cause of MRLS at the time of writing has not been specifically identified, a massive amount of field and experimental information has been carefully accumulated. To critically evaluate these data and provide guidance for future research as well as developing recommendations to prevent the syndrome, a workshop was convened in August of 2002 at the Maxwell H. Gluck Equine Research Center in Lexington, Kentucky. This *Proceedings* provides a comprehensive account of the two-day meeting.

We extend our appreciation to all those who participated, and we gratefully acknowledge those who sponsored the meeting.

David G. Powell
Thomas Tobin
April 2003

Acknowledgments

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We are indebted to the skills and thoroughness of Amy Troppmann in organizing the workshop.

The enormous contributions from staff at Maine Chance Farm, without whom the execution of many of the critical experiments on reproducing MRLS would not have been possible, are recognized and gratefully acknowledged.

Workshop Goals

To review the current state of knowledge on Mare Reproductive Loss Syndrome (MRLS) with particular emphasis on the recent findings associated with the eastern tent caterpillar (ETC) as they relate to the future of the prevention and control of MRLS.

Rationale

The causative agents of MRLS appear to be strongly associated with the eastern tent caterpillar. The causative agents appear to be remarkably specific for the feto-placental unit. At this time, the nature of the agents is unknown. The workshop was designed to review our current knowledge and address the need for future research.

Mare Reproductive Loss Syndrome Case Definition

Two major disease entities have been identified with Mare Reproductive Loss Syndrome (MRLS), which occurred between April and June of 2001 and 2002.

- Early fetal loss (EFL) occurred within 35 and 100 days after mares were bred. In mares that underwent ultrasound examination during this period, fetal death followed by expulsion of the fetus was associated with the presence of abnormal echogenic fluid (cloudy and flocculent) around the fetus.
- Late fetal loss (LFL) occurred as abortion during the last trimester of pregnancy. LFL was associated in many but not all cases with a swollen and engorged placenta (premature placental separation or "red bag" syndrome). Foals born alive were sometimes weak and required intensive veterinary care.
- In addition to reproductive losses, a number of cases of pericarditis (inflammation within the sac surrounding the heart) and severe unilateral uveitis (inflammation of one eye) were associated with the occurrence of MRLS.

Session 1

Field and Clinical Observations on Mare Reproductive Loss Syndrome

Chairperson: Dr. Bill Bernard, Rood and Riddle Equine Hospital, Lexington, Kentucky

Clinical Observations Associated with Early Fetal Loss in Mare Reproductive Loss Syndrome during the 2001 and 2002 Breeding Seasons

W. T. Riddle

I WOULD LIKE TO BEGIN WITH A BRIEF HISTORY OF THE early fetal loss (EFL) syndrome associated with Mare Reproductive Loss Syndrome (MRLS). On April 26, 2001, I ultrasounded six mares on one farm to determine the sex of their fetuses. All mares were between 60 and 70 days of pregnancy. Two of these mares (at 63 and 65 days) were found to have dead fetuses surrounded by allantoic and amniotic fluids containing hyperechoic material. These would be my first cases of MRLS and the beginning of an unprecedented number of early abortions in Central Kentucky. A report released by the Governor's office estimated the number of Thoroughbred EFL caused by MRLS in 2001 to be 2,998 (1).

These large numbers had never before been reported in Central Kentucky nor anywhere in the world. The only comparable experience was in 1980 and 1981 when smaller numbers of EFL were seen in Central Kentucky. Estimates were 256 in 1980 and 162 in 1981 (2). It is difficult to make exact comparisons because ultrasound was not in use at that time, but the breeding dates and the gestational ages were similar. Another similarity was the occasional recovery of fetuses and membranes from the reproductive tract. This is an uncommon finding for abortions at this stage of gestation. No cause was ever determined for the losses in 1980 and 1981.

Mares experiencing EFL from MRLS typically present with no outward signs. Occasionally the mare will present with a sero-sanguinous or purulent vulvar discharge. Some mares may be found with membranes protruding from their vulvas with the fetus located either in the vagina or uterus. A small percentage (estimated at less than 5%) may exhibit mild colic signs, abdominal straining, or low-grade fevers (101-101.5°F) 1 to 3 days before EFL occurs.

Often with MRLS losses, rectal palpation will indicate the mare is pregnant. The uterus may have normal to slightly less fluid distension expected for the stage of gestation, and it is only on ultrasound examination that the mare is found to have a compromised or dead fetus. Prior to the appearance of MRLS in 2001, most mares were not ultrasounded after 30 days of gestation unless fetal sexing was requested. It has now become standard practice for most veterinarians in Central Kentucky to continue ultrasounding through the 60- to 90-day pregnancy examinations. The typical appearance on ultrasound for an MRLS loss is a dead fetus surrounded by an echogenic allantoic fluid and a more echogenic amniotic fluid.

The majority of losses in my practice have occurred between 40 and 80 days of gestation, with a range of 32 to 140 days (Table 1). In 2001, these mares were bred between February 10 and April 10, while in 2002 breeding dates ranged from February 15 to April 1. In 2001, losses began on April 26 and ended on July 2, while losses in 2002 began on April 29 and ended on June 3. This summer I have had four additional EFL that may have been caused by MRLS, the most recent of which was a mare bred on May 6 that aborted on August 17 at 101 days.

Table 1. Gestational ages represented.

Days	2001	2002
30 - 39	3	0
40 - 49	8	5
50 - 59	11	0
60 - 69	12	5
70 - 79	11	6
80 - 89	4	0
90 - 99	4	1
100 - 140	3	1

The majority of losses in 2001 were concentrated over a three-week period beginning April 26, while losses in 2002 were more evenly distributed over a five-week period beginning April 29. In 2001, I had 56 losses on the seven farms that I service. In 2002, I had 18 losses that occurred on only two of the seven farms. These figures indicate a 68% decrease in MRLS losses from 2001 to 2002. The majority of equine practitioners in Central Kentucky experienced a similar decrease.

Conception rates in 2001 and 2002 were not adversely affected by MRLS. Mares that were less than 30 days pregnant during the period of exposure were apparently not affected. There was no increase in the normal pregnancy loss rate between 15 and 30 days gestation.

In 2001, the status of the mares that aborted in my practice was as follows: barren, 19 (34%); maiden, 20 (36%); and foaling, 17 (30%). Thus barren, maiden, and foaling mares were roughly equally represented. In 2002, the status of the mares that aborted was as follows: barren/aborted, 5 (28%); maiden, 10 (56%); and foaling, 3 (16%). Maidens represented 56% of losses in 2002, which is likely the result of heavy eastern tent caterpillar (ETC) loads in fields containing maiden mares on one farm.

In 2001, serum progesterone levels measured by enzyme linked immunosorbent assay (ELISA) from 10 mares taken when EFL was found averaged greater than 4 ng/ml.

Rood and Riddle Equine Hospital, Lexington, Kentucky.

In 2002, serum progesterone levels from six mares at the time of loss averaged 5.9 ng/ml. Therefore, progesterone levels were adequate to maintain the pregnancies at the time of the abortions.

During 2001 and 2002, uterine cultures were taken on all mares following abortion. Results included *alpha Streptococcus*, *beta Streptococcus*, *Escherichia coli*, *Enterobacter cloacae,* and no growth.

Uterine cytologies taken within 7 days of abortion from five mares in 2001 showed moderate to severe inflammation. This inflammation is consistent with recent abortion and is not unique to MRLS. Following abortion, all mares were lavaged and treated for 5 days with the appropriate antibiotic based on culture and sensitivity. On subsequent heats, most mares had normal cytologies and no growth on uterine culture. In 2001, uterine biopsies performed on 10 mares one to three months after MRLS abortion showed no significant pathology. There were no changes on biopsies that could be related to MRLS.

Of the 56 mares that aborted from MRLS in 2001, 31 remained in my care in 2002. Thirty of these 31 conceived, and one mare remained barren. Two of the 30 pregnant mares were again affected by MRLS on their subsequent pregnancy in 2002.

In 2001, aspirates of allantoic fluid were taken by pipette through the cervix from three mares with dead fetuses *in utero*. Cultures of this fluid grew *alpha Streptococcus* in two cases and *E. coli* in the third. Cytologies of these fluids showed sheets of squamous epithelial cells, cocci in chains, and a rare neutrophil. Based on this finding, it is likely that the hyperechoic material seen on ultrasound is squamous epithelial cells.

Complete blood counts and chemistries taken from three mares at the time of abortion in 2001 showed no significant abnormalities.

The appearance of hyperechoic allantoic and amniotic fluids in mares affected by MRLS has been a consistent finding. Prior to 2001, during thousands of fetal sexing ultrasounds of pregnancies between 58 and 75 days, the presence of echogenic fluids was not reported. In 2001 and 2002, all mares in which the dead fetus was found *in utero* had hyperechoic fluids. Many other mares were also found to have echogenic fluids with a live fetus. In 2001, the appearance of this fluid prior to day 80 was associated with a greater risk of loss. A review of May 2001 records in my practice shows 29 mares that were less than 80 days of gestation with live fetuses and echogenic fluids. Of these 29 mares, eight (27.5%) aborted within 30 days. This can be compared to May 2002 records that show 69 mares less than 80 days with live fetuses and echogenic fluids. Only five (7%) of these mares subsequently aborted. This may be related to a decreased exposure to the MRLS insult.

In 2001, because hyperechoic fluids were associated with MRLS, the appearance of this echogenicity was cause for concern. All mares were followed closely by ultrasound, and as pregnancies reached 80 to 90 days, all pregnancies developed echogenicity. While there was concern that this was an indication of exposure to the MRLS insult, it was known that in later pregnancy (150 days plus) hyperechoic fluids were normal.

A study was designed to determine when reproductively normal mares develop echogenic fluids and to determine if the fetal fluids of mares exposed to MRLS differed from the fetal fluids of mares not exposed (3).

One hundred seventy-eight mares that were between 55 and 176 days of gestation were evaluated between July 30, 2001, and August 30, 2001. One hundred four of these mares were in Kentucky, and 74 were in Florida. The results showed that in both the Kentucky and Florida mares the allantoic and amniotic fluids before 85 days are anechoic, and after 85 days both allantoic and amniotic fluids have hyperechoic material.

By mid-June of 2001, echogenic fluids were seen only rarely in pregnancies less than 80 days. In 2002, the number of mares with echogenicity before 80 days also declined by mid-June but remained higher than in 2001. During August 2002, there was an increase in the percentage of mares with echogenicity before 80 days. No EFL was associated with this increased echogenicity.

When MRLS was first identified in 2001, there was an obvious desire to implement treatments or control measures aimed at preventing further abortions. It was very difficult to make reasonable recommendations because the cause of the syndrome was not known.

In my practice, the following treatments were suggested for mares considered at risk:

1. Sulfa-trimethoprim and other broad-spectrum antibiotics—Because bacteria had been cultured from aborted fetuses and placentas.
2. Domperidone—Because some of the late fetal losses (LFL) showed some signs consistent with fescue toxicity (premature placental separation and thickened placentas).
3. Mycotoxin binders—These were used in case there was a fungal pathogen.
4. Flunixin meglumine—This was used for its anti-inflammatory and anti-endotoxin functions.
5. Pentoxifylline—This was recommended for its reagenic properties.

In hindsight, these treatments did not appear to be of any help. Neither Regumate or progesterone was recommended because levels in aborted mares had not indicated a progesterone deficiency.

When considering the cause for MRLS, my clinical impression strongly supports ETC as the cause. In 2002, efforts at prevention were focused on limiting pasture turnout and reducing or eliminating exposure to ETC. Most farms attempted to control caterpillars by either spraying or cutting down cherry trees. When spraying was successful at eliminating caterpillars, MRLS was prevented; however, in many cases sprays were not effective. Farms that cut down all cherry trees reported minimal losses to MRLS. In addition to caterpillar control, limiting turn-out and muzzling were the two most common methods of prevention employed. Many farms were successful at preventing MRLS by limiting turn-out to 2 to 6 hours a day; however, there were farms that limited turn-out that had significant losses. Muzzling mares while on pasture appeared to be close to 100% at preventing MRLS.

I would like to conclude with a short video showing a 63-day pregnancy and the dramatic changes in the allantoic and amniotic fluids that occur following experimental exposure to ETC. These changes occurred over a 24-hour period in a mare that had been tubed with ETC 7 days earlier. These fluids are consistent with fluids from clinical cases of MRLS. I am convinced the ETC plays a direct role in MRLS and now strongly recommend aggressive elimination of the caterpillar.

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Field and Clinical Observations Related to Late Fetal Loss in Mares Affected with Mare Reproductive Loss Syndrome

S. Brown

THE OCCURRENCE OF LATE FETAL LOSSES (LFL) ASSOCIATED with the Mare Reproductive Loss Syndrome (MRLS) ranks second in number of cases affecting the pregnant mare population in the Central Kentucky area in the spring of 2001 and 2002. While staggering and certainly tragic are the losses sustained by area horsemen related to the early fetal losses (EFL), this clinical entity can present with catastrophic outcomes from its effect on both the mare herself and the presenting foal. Managers, owners, and veterinarians were faced with cases numbering more than 500 in the spring of 2001 present from late April through early June. The 2002 season also saw a return of LFL cases but to a lesser degree with approximately 150 suspected cases (information supplied by the University of Kentucky Livestock Disease Diagnosis Center [UKLDDC]). This time frame of occurrence of the LFL cases corresponds with the EFL findings and supports a common insult that most believe is playing a role in this syndrome. The occurrence of these LFL cases has also correlated clinically with the exposure of mares to eastern tent caterpillars (ETC).

Clinically, these mares would present to local horsemen with a definitive set of clinical signs. The mares would show symptoms of restlessness, discomfort, and sweating followed by an intense and explosive presentation with attempts to deliver their respective foals. Most often, individuals would present with premature placental separation with the appearance of the engorged chorioallantoic membrane ("red bag" syndrome). The presentations were mostly compromised by abnormal foal positioning resulting in difficult dystocias with mares in significant distress oftentimes reported to be reluctant to foal in a recumbent position. Some of these mares failed to reach full mammary development or achieve "waxing" prior to these explosive deliveries. Veterinary intervention was often necessitated in these cases so appropriate measures could be implemented to avoid losses for both the mare and foal. If delivered alive, these neonates were compromised as weak, dehydrated, and dysponeic requiring immediate efforts to achieve oxygen supplementation while en route to area referral hospitals. The placentas from these mares were grossly heavy and edematous, weighing as much as 40 pounds, with umbilical cords showing signs of ecchymotic and petechial hemorrhages on their surfaces. Some mares would present with normal deliveries with evidence of amnionitis with yellowing and edematous formations on the placental membranes. Microbiological assays

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routinely yielded evidence of *alpha-hemolytic streptococcus* sp. and *Actinobacillus* sp., among other organisms.

The question to be addressed revolves around the nature of the toxic insult responsible for the pathological processes identified in these mares and their foals. Initially, cases included "red bag" presentations typically associated with exposure to endophyte-infested tall fescue. These mares did not present with prolonged gestations as with fescue toxicosis, and the placentas from these mares were intensely more inflamed than those typically seen with fescue. Thus, attempts at preventative treatment with domperidone resulted in little benefit to these mares in protecting them from the effects of this syndrome.

Pathophysiologically, evidence of LFLs in these mares centered around some common associations. There was an intense placentitis at time of foaling associated with thickened placental membranes. Funisitis associated with the amniotic section of the umbilical cords of these neonates was observed with ecchoymotic and petechial hemorrhage on the surface. Microbiologically, the consistent ability to recover *alpha-hemolytic streptococcus* and *Actinobacillus* sp. from umbilical tissues, lungs, and placentas of affected individuals has become almost a hallmark for defining the clinical entity of LFLs. From these findings, we must attempt to elucidate the nature of the inciting insult and the duration of exposure to cause an abortion. The presence of these microorganisms specific to this condition has caused speculation that an underlying immunologically compromised situation allows the bacteria to flourish as commensals.

Many practitioners in the spring of 2001 and 2002 made numerous attempts to provide useful clinical data by monitoring the development of these cases and in an attempt to identify potential benefits of therapeutic intervention. Ultrasonography, both trans-rectal and trans-abdominal, has been utilized by veterinarians to measure placental thickness and separation at the region of the cervical star, as well as evaluation of fetal parameters such as fetal heart rate to measure fetal stress. Hormonal assays may also be an area of potential benefit to measure placental function. Treatment centered on broad-spectrum antibiotic therapy (trimethoprim-sulfa, nonsteroidal anti-inflammatory drugs [NSAIDs], pentoxyfilline, and vitamin E/selenium) used systemically in supporting the compromised placental unit to improve foal survivability. Future studies of LFL incorporating these monitoring techniques may provide beneficial information to place in the hands of practitioners and horsemen in dealing with these cases.

As with the other clinical manifestations of MRLS, the LFL cases present all of us, veterinarians, horsemen, and researchers, with a number of perplexing and unexplained questions related to these findings. The timing of these cases, both in season of the year and in association with EFL, makes many ponder if there is a dose-dependent response nature related to the insult or a multifactorial nature that translates into the different clinical scenarios. Some cases with remarkable similarity have been reported earlier in the season. There are reports of LFLs extending into southern hemisphere foaling mares in both 2001 and 2002 during late July and August. Again, is there something unique about these reported sporadic occurrences, and how do they relate to the bulk of normally occurring cases? It will be our endeavor to reach a greater understanding of how all these inconsistencies "fit" into a plausible explanation as we come to fully understand the pathophysiology of MRLS.

Clinical Observations of Mare Reproductive Loss Syndrome in Critical Care Mares and Foals

T. D. Byars and T. L. Seahorn

AT THE END OF APRIL AND THE BEGINNING OF MAY IN 2001, a significant increase in deceased or clinically compromised newborn foals was presented to the University of Kentucky Livestock Disease Diagnosis Center (UKLDDC) or to Central Kentucky equine veterinary neonatal intensive care units, respectively. These pathologic and clinical presentations were observed in an acutely profound increase in caseload numbers with the eventual designation as the Mare Reproductive Loss Syndrome (MRLS). Prodromal signs in mares were inconsistent but varied from being gestational term to within 30 days of foaling, asymptomatic or as depressed and self-isolated in the pastures, having muscle tremors, an implied lack of mammary development due to a rapid onset of foaling, clinical episodes similar to colic, and a rare stiffness that resembled acute laminitis.

The majority of mares lacked clinical signs of an impending foaling and were not identified as "high-risk pregnancies," and agalactia was assessed as present only by the mare's physiological preparation for foaling being acutely compromised. Foalings often occurred without rec-

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ognition of stage one of parturition with a cluster of stage 2 and 3 being most often simultaneous. An increased number of mares foaled while standing. Premature separation of the placenta was common, and fetal membranes were often passed with the foal (placenta privia or "red bag" presentation).

Foals were consistently developed in size and maturity synchronous to the time frame of the gestation. Placentas did not reveal consistent gross abnormalities, with the exception of funisitis found in a significant number of umbilical cords at the UKLDDC. Post-foaling mares were treated with conventional uterine lavage, and a failure to conceive during 2001 was not a feature in this population of mares as opposed to mares experiencing early embryonic losses. The use of prophylactic medications in foaling mares did not provide an apparent resolution in the abnormal foalings from farms experiencing large numbers of losses. These subjective treatments included antibiotics, pentoxifylline, and domperidone.

Clinical signs in the neonates were significantly associated with perinatal asphyxia, requiring resuscitation at birth. Most foals admitted to the intensive care units were dehydrated with signs consistent with the systemic inflammatory response syndrome (SIRS) or sepsis. Hypothermia, tachycardia, and inconsistent respirations were persistently noted in hospital admissions, often with periods of apnea leading to respiratory arrest. Blood cultures were rarely positive, although *alpha Streptococcus* and *Actinobacillus* sp. were the most frequently isolated organisms. Clinical pathology consistently revealed severe dehydration and a neutropenia (400 to 2,500 white blood cells/mm at admission). Surviving foals had relatively higher white cell counts, albeit below normal, or the foal showed a more rapid rise to normal neutrophil numbers during the first 2 days of hospitalization. Hypoglycemia was most present, often below 20 mg/dl. Colostral transfer was consistently inadequate with most immunoglobulin G (IgG) levels approximately 150 mg/dl at 24 hours of age with the use of donor colostrum and, in some cases, following the use of intravenous plasma. The hypoxemic-ischemic (HIE) syndrome occurred in most foals as comatose "sleepers" but rarely with either pre- or post-coma seizure activity. The prognosis for survival was associated with improved blood values and foals exhibiting normal respiratory patterns and rates.

Survivals varied from initially having a high mortality rate to an eventual improved prognosis without noting marked changes in the treatment regimes. Treatments were empirical and subjective principally utilizing routine antibiotics, plasma, fluid therapy, enteral and/or parenteral nutrition, vitamin E, dimethyl sulfoxide (DMSO) or mannitol, anti-inflammatory medications often with single doses of corticosteroids, oxygen supplementation, and intensive nursing care. The variables for survival and differentiation from conventional septic foals included the actual gestational length, a decrease in the "toxic" insult within 1 to 2 days, and the subjective improved clinical care and therapeutics. Surviving foals did not have residual impairment.

The MRLS syndrome for foaling mares and their foals should not be considered apparent in individual patients since a pattern of abnormalities must be present in larger numbers for a more definitive diagnosis. In our experience, individual cases could not be separated from the more "routine" septic foals provided annually in a dense population of high-risk mares. The epidemiological appreciation for the consistency of clinical scenarios provided with an abrupt and significant increase in neonatal caseload may be presumptuous for MRLS, but a single patient presentation would not be sufficient to declare MRLS in a foaling mare. However, clinicians should be astute in evaluating severely compromised foals without dysmaturity or obvious placental abnormalities from mares that acutely foal without the conventional stages of parturition as being suspicious for MRLS. The appreciation for a presentation of a peracute and seemingly toxic insult to foaling mares with the respectively clinically ill foals in increased numbers beyond a seasonal norm should signal the preliminary diagnosis of MRLS until other causes have been ruled out. The progressive seasonal increase in survival may be a reflection of a singular environmental insult that decreases in susceptible mares.

Endophthalmitis: A Syndrome Associated with Mare Reproductive Loss Syndrome?

C. Latimer

THIS PRESENTATION WILL SUMMARIZE MY OBSERVATION OF equine endophthalmitis cases in Lexington, Kentucky, during the outbreak of Mare Reproductive Loss Syndrome (MRLS) in 2001 and 2002. The numbers represent my patients only. Twenty-four cases were seen in 2001, and 11 were seen in 2002. Except for the decreased incidence in 2002, no other feature distinguished the two years, so the data are combined.

Two-thirds of the horses presented in the first two weeks of May, with further cases scattered until June 8. Breeds affected seemed to reflect the local population, with Thoroughbreds accounting for 26 of the 35 cases. Males and females were almost equally represented, 19 males, 16 females. Fifteen yearlings and six foals were affected, with one or two horses of 2, 3, 4, 5, 6, 9, 10, and 12 years of age.

Clinical presentation was characterized as an acute onset, unilateral, exudative endophthalmitis. Horses consistently exhibited corneal edema and exudate in the anterior and posterior segments. Variable degrees of pain, periocular swelling, discharge, and hyphema were observed. Diagnosis was based on ophthalmic examination. Bloodwork performed to evaluate status for *Leptospirosis*, *Blastomycosis*, *Histoplasmosis*, equine herpesvirus (EHV), influenza, and *S. equi* was non-diagnostic. Culture and cytology of aqueous and vitreous samples were unproductive. Ultrasound examination revealed a turbid vitreous.

Treatments included systemic and topical antibiotics, anti-inflammatories (NSAIDs and corticosteroids), tissue plasminogen activator, atropine, mycotoxin binders, and cyclosporine. Response to treatment was poor, with 34 of 35 eyes blind and "mid-phthisical." One horse was visual after prolonged systemic corticosteroids and antibiotics, but it is not possible to be certain that this patient was a member of the MRLS/endophthalmitis group, as his symptoms were typical but mild.

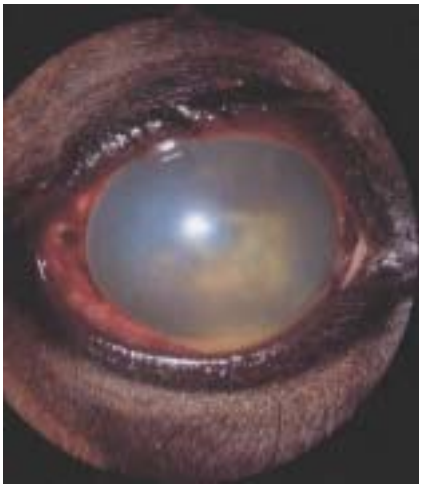

Figure 1. 3-month-old Thoroughbred filly. Corneal edema and anterior chamber debris obscure the iris.

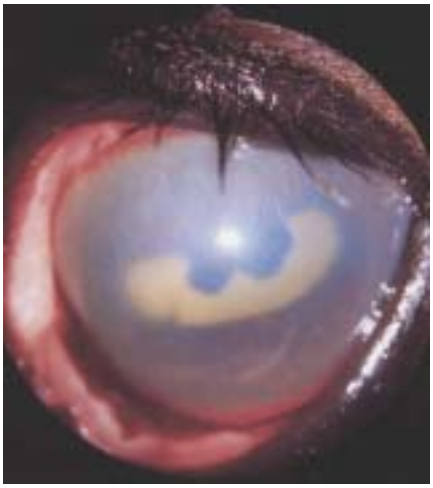

Figure 2. 10-year-old Thoroughbred mare. Typical appearance of the anterior segment. In patients with a visible pupil, the vitreous was noted to be opaque.

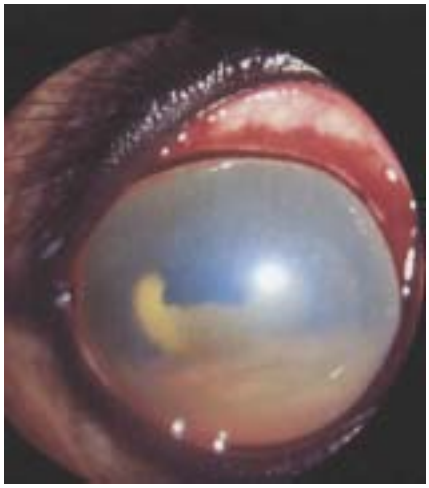

Figure 3. 1-year-old Thoroughbred colt. Some hemorrhage is seen in the anterior segment debris.

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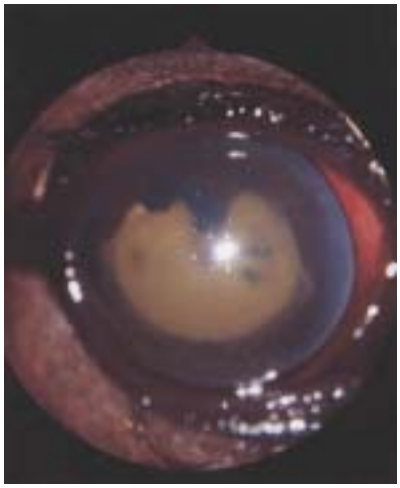

Figure 4. 1-year-old Thoroughbred colt. This colt made some response to initial treatment (e.g., pupillary dilation), but within 48 hours the iris was no longer visible.

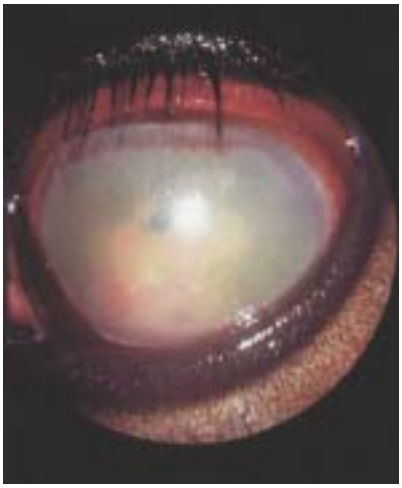

Figure 5. 1-year-old Standardbred filly. Note the limbal vessels, marked corneal edema, and obscured anterior segment.

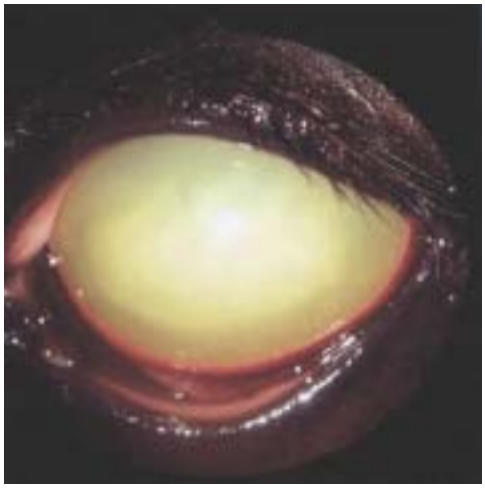

Figure 6. 2-year-old Thoroughbred colt. Uniform corneal edema, xanthochromic diffuse anterior chamber debris, and active limbal vessels are seen.

Important Questions about This Group of Horses

1. What pathogenesis can explain a 100% unilateral occurrence?
2. Why were yearlings and young horses over-represented in the affected population (15 of 35 yearlings and 28 of 35 <4 years old)?
3. Why did the endophthalmitis cases not have other symptoms of MRLS (pericarditis, EFL, LFL)?
4. Why didn't patients respond to treatment for infection or inflammation?

If these endophthalmitis cases are related to MRLS, as strongly suggested by their temporal occurrence, answers to the above questions may elucidate the pathogenesis of this syndrome.

Clinical Observations of the Pericarditis Syndrome

N. M. Slovis

THE PERICARDIUM OF THE HORSE CONSISTS GROSSLY OF TWO pericardial layers, with appropriate nerves, blood vessels, and lymphatics. The visceral pericardium directly covers the heart surfaces and is sometimes referred mistakenly as the "epicardium." The parietal pericardium is commonly referred to simply and confusingly as the "the pericardium." The parietal pericardium is lined by mesothelial cells, varies in thickness from 1 to 3.5 mm and contains serous fluid that is considered as an ultrafiltrate of the blood plasma (1). The physical effect of the pericardium is to restrict overall heart filling. It helps limit acute cavitary over distension following ruptured papillary muscle, pulmonary hypertension, and acute myocarditis. The pericardium also offers atrial support against ventriculoatrial regurgitation as well as distributing hydrostatic forces over the surface of the cardiac chambers. The mesothelium has cyclooxygenase, prostacyclin synthetase, and lipoxygenase activities. Large amounts of prostacyclin are continually released by the entire pericardial mesothelium into the pericardial cavity in response to pericardial stretching, to increases in myocardial work and loading conditions (1). Prostacyclin influences the tone of the underlying coronary vessels but also has fibrinolytic activity with hemopericardium.

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Pericarditis or inflammation of the pericardium is relatively uncommon in horses; three forms have been described including effusive, fibrinous, and constrictive (2-11). Causes of equine pericarditis include immune-mediated diseases, vasculitis, disease of contiguous structures, trauma, bacterial and viral infection, and neoplasia; however, for many cases, the etiology remains undetermined. While most reports in the literature describe the clinical presentation, treatment. and outcome of a single case, two retrospective studies reported a series of cases, providing relevant prognostic information (10-11).

During the spring of 2001 and 2002, a larger than usual number of cases of fibrinous pericarditis was observed in Central Kentucky. During late April and May of 2001 and 2002, 33 and six horses, respectively, were presented to our clinic with effusive, fibrinous pericarditis. The most common primary complaints were fever, azotemia, colic, weight loss, anorexia, lethargy, tachypnea, and tachycardia. Nineteen of the horses were greater than two years of age, 12 were between one and two years of age, and eight were less than one year of age. Physical examination revealed tachycardia (n = 35), pyrexia (101.5 to 104.8° F) (n = 29), muffled or inaudible heart rate (n = 27), lethargy (n = 24), tachypnea (n = 23), jugular pulses or distension (n = 23). Echocardiograms were performed, and all 39 patients had effusive, fibrinous pericarditis; 18 (45%) of the horses had evidence of right-sided heart failure characterized with pleural effusion and/or ascites.

Diagnostic test results revealed a leukocytosis in 21 cases. Hyperfibrinogenemia was detected in 25 horses. Cardiac troponin I was normal in 3 of 3 horses. Equine viral arteritis (EVA), equine herpesvirus (EHV-1, EHV-2, and EHV-4), and influenza titers were performed, and no significant rise in titers was noted. Polymerase chain reaction (PCR) for EHV-1, EHV-2, and EHV-4 was evaluated on six transtracheal washes (TTW) and were negative. An influenza ELISA test was performed on six TTW samples and was negative. Mycoplasma was submitted for culture in nine pericardial samples and one TTW sample. All were negative. Histoplasmosis titers were measured in three horses and were negative. TTW were performed on six horses. Five of six samples isolated a pathogen consisting of *E. coli* (2), *alpha Streptococcus* (1), *Actinobacillus* and *Pasteurella* (1), *Rhodococcus equi* (1), and *Enterobacillus cancerogenus* (1).

Thirty-eight horses had to have a pericardiocentesis and drain placement to allow evacuation of the pericardial effusion. Thirteen of the horses had pericardiocentesis performed one time, 12 had the procedure performed twice, four had three procedures, and one had the procedure performed five times. The amount of fluid evacuated from the pericardial space ranged from 1 to 22 liters. Laboratory analysis of the fluid in two-thirds of the cases was characterized as a sterile exudate with a cell count ranging from 900 to 28,000 white blood count (WBC)/MM and a total protein count ranging from 3.3 to 5.4 g/dl. A third of the cases had a septic exudate from which *Actinobacillus equuli* (5), *Streptococcus* spp. (2), *Pasteurella multocida* (1), *Staphylococcus aureus* (1), *Acinetobacter* (1), and *Pseudomonas* (1) spp. was isolated.

Treatments consisted of intrapericardial administration of corticosteroids (20 to 50 mg dexamethasone or 100 mg solu-delta-cortef or 30 mg triamcinolone acetonide), antibiotics (ceftiofur, ampicillin, ticarcillin, and/or potassium penicillin), heparin (5,000 IU), hyaluronic acid, and/or streptokinase. All 39 horses were treated with systemic antibiotics (potassium penicillin/gentocin, ceftiofur, tribrissen), NSAIDs (phenylbutazone or flunixin meglumine), corticosteroids (dexamethasone 6 mg to 50 mg intravenous (IV) once a day (SID), furosemide, heparin, colchicine, pentoxifylline, intravenous fluids, vitamin E, and antiulcer medication (omeprazole).

Six horses (15%) died one month to one year after the initial diagnosis of pericarditis. Five were euthanized either because of finances or because of a poor prognosis. Retrospectively, horses less than one year of age were noted to have a poor prognosis for long-term survival. Five of the eight horses less than one year of age were either euthanized or died secondary to pericarditis. Three patients had two or more reoccurrences, while four had one reoccurrence of effusive pericarditis. One of the patients that had one reoccurrence won its maiden allowance race one year after being diagnosed with pericarditis.

A case-control study was undertaken to identify factors associated with an epidemic of equine fibrinous pericarditis (EFP) cases that occurred concurrently with the Mare Reproductive Loss Syndrome (MRLS) (12). Thirty-nine horses that developed EFP during the spring of 2001 and 30 control horses were studied. Distribution of times of diagnosis of EFP was consistent with a point-source epidemic. Results indicated that occurrence of MRLS and EFP were related. Exposure to eastern tent caterpillars (ETC) emerged as the strongest risk factor for development of EFP. Although causality could not be definitively determined for any of the factors studied, results indicate that methods for reducing exposure to ETC would be expected to reduce risk of development of EFP.

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Summary

M. LeBlanc

I'D LIKE TO GIVE A SHORT SUMMARY AND FINISH OFF WITH some of my thoughts on reproductive physiology and take the liberty in asking some questions and providing some scientific information that maybe supports some of the questions and data that I'll present.

First of all, Dr. Riddle started the session by stating that a similar scenario occurred in 1980-1981. The number of cases was much lower. We didn't have ultrasonography at that time. However, *alpha Streptococcus* and *Actinobacillus* sp. were recovered, similar to what was seen in 2001 and 2002. Four scenarios occurred in 2001 and 2002, the first being early fetal losses (EFL). In 2001, there were approximately 3,500 EFL. This year, although I don't have the exact numbers, it appears it has decreased to less than a third of what was seen in 2001. The second clinical scenario was the late fetal losses (LFL), with the dramatic and severe foal losses occurring over a short time frame. These data were presented by Dr. Brown and Dr. Byars. Last year there were 500 losses, and this year it appears that there were about 150 losses.

There were two scenarios seen in young and older horses. The first was pericarditis, which occurred in animals of all ages, although there were 20 cases in horses less than two years of age. The other was endophthalmitis, which was much more predominant in younger horses than older horses, especially considering the population and ages of the mares and stallions in the area.

Pregnancy losses and clinical disease occurred in late April to early May, and it correlated and was possibly associated with the rise and fall of eastern tent caterpillars (ETC) as they molted and continued to molt and then wandered along the fencelines and onto pastures.

In 2001, the length of time pregnancy loss and clinical disease occurred was approximately three weeks, whereas this year we saw it over five weeks.

So the big questions that we're here trying to answer are: What is the toxin? Where is the toxin coming from? And, if we can identify the toxin, how do we treat it?

Are there any similarities between these clinical syndromes? I believe there are, but I'm sure there will be people who question my thought process.

Is there a relationship between EFL and LFL? There appears to be. From Dr. Riddle's data, it appeared that the greatest losses in his clinical practice occurred between 40 to 80 days of gestation. This is the period of time when the placenta is attaching. The equine placenta attaches between 40 to 100 days of gestation, but it is quite tightly attached by 80 days (1).

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Are the hyperechoic particles in the allantoic fluid and possibly in the amnionic fluid sloughed off cells from the trophoblast or placenta? Are these cells sloughing because placental attachment cannot occur? Is the placenta more susceptible at this period of time, or, as Dr. Riddle said, is it due to the fact that we don't have many mares pregnant between 120 days and nine months gestation during that period of time, so we don't see clinical disease?

However, there are a lot of warmbloods that do not foal until June or July that live in southern Illinois, Ohio, and possibly in North Central Kentucky. Were these mares affected in that time range, because they're not going to foal until June or July?

Is the equine placenta more susceptible to early detachment after nine months of gestation? Dr. Peter Rossdale in Newmarket in the early 1980s did some classic experiments where he looked at effects of oxytocin versus prostaglandin on early detachment of the placenta and birth of the foal. In those studies, he showed that the mare will respond and deliver a foal with one injection of oxytocin as early as 9½ months of gestation. However, she will not respond to repeated injections of prostaglandin in that she will not deliver until the placenta or the fetus is ready. So, in late gestation, is the toxin or the insult causing early detachment of the placenta? This early detachment of the placenta renders the foal in a perilous situation where it's not receiving any more oxygen. We have a hypoxic-ischemic insult, with possible placental detachment, placental edema, and the foal cannot breathe. Delivery of the foal in these mares is dramatic. The mare is trembling, there is premature placental separation, and an explosive birth. Many times the mare delivers while standing. Are the two events related—early fetal and late gestational losses? Are the placentas early in gestation and late in gestation affected either by a toxin that does not allow it to attach in early gestation, whereas in late gestation the toxin induces early and sudden detachment?

The bacteria commonly isolated were *alpha Streptococci* and *Actinobacillus* sp. We rarely see those in early placental problems or in early fetal reabsorptions, nor do we see them late in gestation. So how do these bacteria get in, how are they causing the insult, and are they a primary or a secondary problem? Does the toxin carry the bacteria into the pregnancy late in gestation? Why do we see funisitis? Why do we see amnionitis? Why is primarily a respiratory infection leading to possible pneumonia and death in these foals?

Two syndromes didn't primarily affect pregnant mares, endophthalmitis and pericarditis: Are these immune-mediated diseases? Does the disease occur in younger horses, not because of contact, but because of an immune-mediated complex? Why do dogs get blue eye when they are vaccinated against leptospirosis? From my memory, when I was in small animal practice in the 1970s, it's usually unilateral. Different scenario, but it is a unilateral disease. Is the pericarditis an immune-mediated response to either the toxin or to the bacteria that eventually get into the pericardial sac? I can't answer these questions, and hopefully in the next 48 hours we can come up with some answers.

One last comment about the immune system: Remember that the pregnant mare has a modulated immune response. She goes from a thymus helper-1 (TH-1) pro-inflammatory cytokine response to thymus helper-2 (TH-2), which is an immune-modulated response. So, she is under a more modulated system than a non-pregnant animal that is more apt to respond with a pro-inflammatory cytokine response or the TH-1 response. Is that immune system causing the two different scenarios that we see?

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Session 2

Laboratory Correlates of Mare Reproductive Loss Syndrome

Chairperson: Dr. Lenn Harrison, Livestock Disease Diagnostic Center, College of Agriculture, University of Kentucky

Gross and Histopathological Correlates of Mare Reproductive Loss Syndrome

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IN THE LATE SPRING OF 2001 AND TO A LESSER EXTENT IN 2002, Central Kentucky horse farms experienced epidemic losses of fetuses and foals and other atypical equine health problems. This group of conditions has been referred to as Mare Reproductive Loss Syndrome (MRLS). During this time, increased numbers of fetuses (early and late fetal losses [EFL and LFL]), placentas, and term foals were presented to the University of Kentucky Livestock Disease Diagnosis Center (UKLDDC) for necropsy examination and diagnostic testing.

Materials and Methods

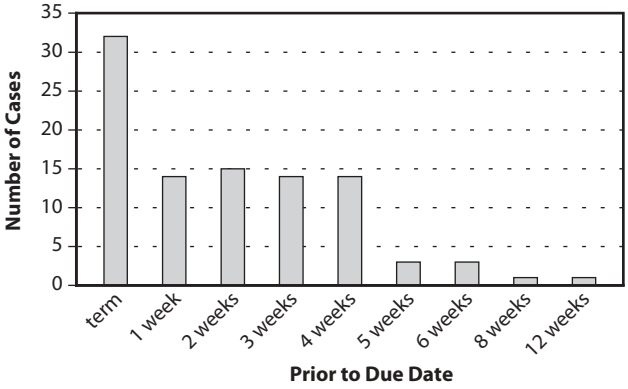
Foals, fetuses, and placentas were necropsied, and samples were taken for microbiology, serology, toxicology, and histopathology. Complete pathological reports were issued on the cases. The findings in 100 randomly selected MRLS LFL cases were reviewed and characterized for this report.

Results

During the 2001 and 2002 MRLS epidemics, approximately 550 and 250 LFL, respectively, were presented to the UKLDDC for examination. Necropsy examination and laboratory testing revealed a number of consistent findings in these fetuses.

Multiple breeds and all ages of mares were affected. Most of the fetuses were delivered at term or were aborted several weeks prior to the due date with usually no premonitory signs in the mares (Figure 1). The delivery was characterized as "red bag" in 32% of the cases, indicating that the allantochorion was presented and passed concurrently with the fetus. Lesions were seen in both the fetus and placenta. The fetuses were of normal size and weight for the gestational age and typically were in a state of good postmortem preservation. The lungs had variable inflation from case to case, ranging from no aeration to moderate aeration, indicating respiratory efforts at the time of delivery. The lungs were sometimes slightly firm, suggesting pneumonia. Hemorrhages were often present on the pleura and heart. Some allantochorions were mildly edematous; however, most were of normal size and weight. Hemorrhages were commonly seen on the chorionic and allantoic surfaces.

Figure 1. Time of LFL relative to due date in 100 randomly selected cases.



The most striking change occurred in the umbilical cord. A high percentage of the cases had roughening of the surface of the cord and enlargement due to stromal edema. There was discoloration of this area, typically a dull grayish-yellow color, and there were stromal and surface hemorrhages. The umbilical cord changes were limited to the amniotic segment with the allantoic portion having the normal smooth, glistening appearance to the surface. The amniotic membranes had variable hemorrhage and edema.

Histopathologically, the lungs often contained numerous desquamated squames of amnionic fluid origin and low numbers of neutrophils and macrophages in the alveolar spaces. Occasionally, multinucleated giant cells were present. Bacteria were often observed in the alveoli. Other fetal tissues contained only variable congestion and acute hemorrhages. Microscopically, the umbilical cords had bacteria on the surface with loss of the epithelium and light to heavy infiltrates of neutrophils and macrophages that were concentrated near the surface of the cord. Hemorrhages and edema were present in the stroma. Similar changes were sometimes present in the amniotic membrane. Allantochorions occasionally had low numbers of neutrophils in the stroma and extraembryonic coelom.

In 2002, cases consistent with MRLS recurred. The temporal, pathological, and microbiological findings were similar to the 2001 epidemic.

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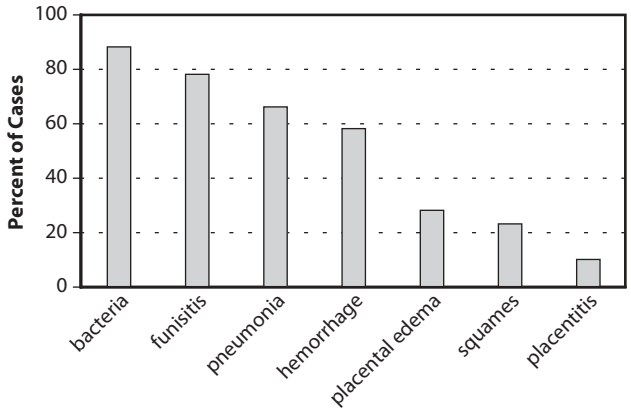
Discussion and Conclusion

The cause of MRLS was not identifiable based on pathological examination; however, a number of disease conditions were eliminated as potential causes. The main pathological findings in MRLS fetuses were inflammation of the umbilical cord (funisitis), pneumonia, bacterial infection, and hemorrhages (Figure 2). The bacterial infection in aborted fetuses represented an *ante mortem* event, and most of the pathological lesions could be attributed to bacterial infection. It could not be determined if the bacteria were primary causes of abortion or merely represented secondary opportunistic infections. The epidemiological findings and bacteria isolated suggest a secondary role for the bacteria. The findings suggest that the bacteria gain access to the amniotic fluid and sites with direct contact with this fluid but show little tendency to invade. The high occurrence of "red bag" delivery, indicating possible premature placental separation, suggests placental injury or problem with placentation.

There was no single diagnostic test or pathognomonic finding that permitted diagnosis of abortion due to MRLS. Diagnosis of MRLS-related abortion or stillbirth was based on a combination of history, time of year, bacteriologic results, and the above-described pathological findings. These pathological findings are suggestive of *in utero* fetal illness, infection, and distress but lack sufficient specificity to allow for diagnosis of a primary inciting cause.

Figure 2. Occurrence of pathological changes.



The Pericarditis Correlate of Mare Reproductive Loss Syndrome

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AN EPIDEMIC OF PRIMARY EQUINE PERICARDITIS OCCURRED in Central Kentucky during the spring of 2001. The outbreak was temporally associated with two other syndromes in horses: early and late fetal loss (EFL and LFL) and uveitis, collectively referred to as Mare Reproductive Loss Syndrome (MRLS). All three conditions were associated with a heavy eruption of the eastern tent caterpillar (ETC), *Malacosoma americanum*, which was later identified as a risk factor in MRLS and pericarditis (3).

During the outbreak, tracheal washes, pericardial fluids, and serum samples from 13 clinical cases and 22 horses with pericarditis were submitted to the University of Kentucky Livestock Disease Diagnosis Center (UKLDDC) for diagnostic testing. Horses submitted for postmortem examination came from 22 farms and nine counties located in Central and Eastern Kentucky (Figure 1). The majority of submissions were Thoroughbreds. Quarter Horse, Rocky Mountain Spotted, and Hanoverian breeds were also represented. Two horses were of unknown or mixed breeding. The average age was 6.4 years with a range extending from three weeks to 30 years. Fifteen were female and seven male.

Pathologic Observations

Significant laboratory findings in the necropsy cases included lesions of effusive fibrinous pericarditis that were typically associated with varying degrees of pericardial, pleural, and peritoneal effusion. Twelve cases had received prior treatment for pericarditis. Recurrent effusions and constrictive pericarditis occurred in six individuals. Microscopically, the epicardium was covered by a thick, moth-eaten layer of fibrin within which were focally dense and diffuse accumulations of neutrophils. Bacteria were occasionally observed. The epicardium was thickened by granulation tissue of varying maturity. Myocardial lesions were generally mild and infrequent and included superficial interstitial fibroplasia and/or fibrosis, focal interstitial myocarditis, and foci of myocardial mineralization and necrosis.

Microbiologic Findings

Aerobic cultures of pericardial fluid and/or myocardium from necropsy (22) and mail-in submissions (11 of 13)

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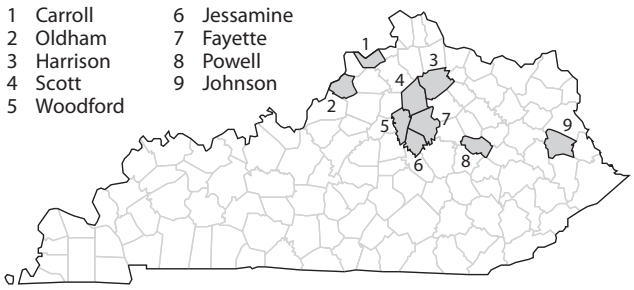
Figure 1. Geographic distribution of pericarditis cases in Kentucky.



yielded an *Actinobacillus* sp. (11), *Streptococcus zooepidemicus* (1), *Escherichia coli* (1), and *Enterococcus fecalis* (1). No bacteria were recovered from the remaining cases. Cultures for mycoplasma organisms were negative. No viruses were isolated in cell culture, and EHV-1 antigen was not detected in tissue by immunofluorescent methods. PCR tests for EHV-2 nucleic acid were negative except in four cases.

The inability to isolate virus from samples submitted from terminal and clinical cases of pericarditis was neither an unexpected finding nor does it exclude previous virus infection as a contributing factor in these cases. In the terminal cases, the inflammatory process in the heart and pericardium had progressed to the chronic stage. It is also likely that at the time of diagnosis most of the clinical cases were subacute to chronic in duration. Although the inability to isolate virus was not considered evidence against viral myopericarditis, epidemiologic findings and observed microscopic alterations in heart and pericardium are not supportive of a viral etiology.

Discussion

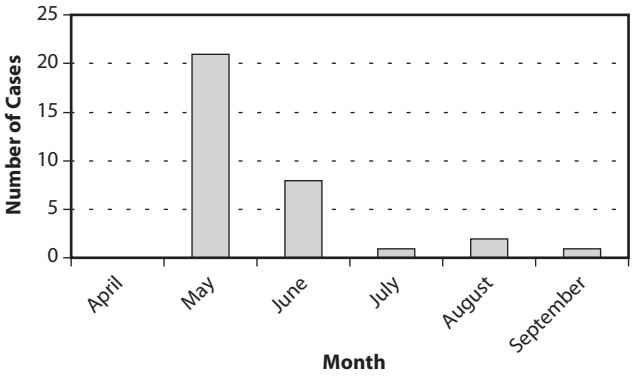
Although an etiology of the pericarditis syndrome seen in this series of horses has not been identified, epidemiologic evidence suggests a point source exposure to an environmental agent and an apparent central causative role of the ETC (3). Laboratory findings support a role for opportunistic bacterial infection in the pathogenesis of the condition but does not exclude the possibility of previous virus infection or toxic effect from an environmental or caterpillar-associated agent.

The cases were presented during a five-month period extending from May through September (Figure 2). The terminal cases submitted after June were horses that had been treated for pericarditis for two to three months and died subsequent to the development of recurrent effusions or constrictive pericarditis. The large number of cases of equine pericarditis occurring over an extremely short period of time in this report is in sharp contrast to all previous reports that have described fewer cases collected over a period of months to years (1,2,4,5,6,7).

All of the terminal nontreated and 36% of the clinical cases were diagnosed as bacterial pericarditis on the basis of positive culture results. Bacteria isolated were similar to isolates reported in earlier studies and included *Actinobacillus* sp., *Streptococcus zooepidemicus*, and *Enterobacteri faecalis*. There is evidence to suggest the remaining cases of terminal pericarditis were also bacterial in origin. The gross and microscopic alterations in the parietal pericardium and heart of affected individuals were similar in all cases and consistent with a bacterial etiology.

Further evaluation of information for each pericarditis case is being undertaken. The additional retrospective investigation is being carried out to attempt to clarify the role of microbiologic agents in this outbreak.

Figure 2. Distribution of pericarditis cases by date of accession.



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Bacteria Associated with Mare Reproductive Loss Syndrome: Late Fetal Losses

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AN ABORTION OUTBREAK AFFECTING THE EQUINE POPULATION of Central Kentucky commenced in the last week of April and extended through May in 2001 and in 2002. During these time periods, there were dramatic increases in the number of early and late fetal losses (EFL and LFL) referred to as Mare Reproductive Loss Syndrome (MRLS). The losses have been associated epidemiologically with exposure of mares to the eastern tent caterpillar (ETC), *Malacosoma americanum*, but the cause of MRLS has not been identified.

After examining tissues (especially lung and umbilical cord) from the LFL received during the epidemic, pathologists at the University of Kentucky Livestock Disease Diagnosis Center (UKLDDC) observed lesions compatible with those caused by bacterial infections (1). They also observed bacteria in the fetal lungs and on the surface of the umbilical cord, and bacteria were recovered from most of the fetal tissues. The purpose of this paper is to report the microbiological findings obtained from the LFL obtained during the MRLS epidemic in 2001 and 2002.

Materials and Methods

Animals

All LFL received at the UKLDDC between April 26 and May 31 in 2001 and 2002 were examined in this study. Premature or term foals that had been treated or had been in a veterinary hospital were not included, and fetuses from mares in early gestation (less than six months) were not included.

Bacteriology

Lung, liver, and stomach content of all fetuses and placenta (if submitted) were cultured for aerobic and microaerophilic bacteria. In 2001, only the lung and stomach content from the last 275 fetuses received were cultured, and in 2002 the umbilical cord was cultured when requested by the pathologist. Each tissue was inoculated to a blood agar plate and an eosin-methylene blue agar plate, which were incubated in 8% carbon dioxide (CO_2) at 37°C for 2 to 7 days. Plates were examined daily for the presence of significant bacteria. A bacterium was considered significant if it occurred in pure or almost pure culture in moderate to numerous numbers from at least two sites. All significant bacteria were identified using conventional bacteriological media and methods (2,3).

Kidney and placenta (liver, if placenta was not received) were tested for the presence of leptospires by a fluorescent antibody test (FAT) (4,5) using a polyvalent conjugate (National Veterinary Services Laboratories [NVSL], Science and Technology, APHIS, USDA, Ames, IA). Only the first 129 fetuses received in 2001 and the first 110 fetuses received in 2002 were tested for leptospires by FAT. However, the stomach contents of all fetuses were examined by dark-field microscopy for the presence of leptospires and other bacteria.

Virology

At the UKLDDC, all fetuses were tested for EHV-1 by FAT in 2001 and 2002 and for EVA by FAT in 2002. Virus isolation was attempted on all cases from a tissue pool (lung, liver, spleen, kidney, and thymus) and, if received, from the placenta. The tissues were macerated and centrifuged. Then the supernatant was inoculated onto three cell culture lines: rabbit kidney (RK-13), monkey kidney (VERO), and equine kidney. Equine kidney was used for only 103 cases in 2001 and 93 cases in 2002.

In addition, tissues from 10 MRLS fetuses were also submitted to the NVSL for virus isolation. Those tissues were inoculated onto five tissue culture cell lines, into embryonating chicken eggs, and by the intracerebral route into 3- to 5-day-old mice.

Livestock Disease Diagnostic Center, College of Agriculture, University of Kentucky, Lexington, Kentucky.

Results

Microbiological examinations were completed on 682 cases: 433 fetuses in 2001 and 249 in 2002. Results of bacteriological culture are reported in Table 1. Significant bacteria were recovered from 510 (74.8%) of the 682 fetuses. The most frequently isolated bacteria were non-beta-hemolytic streptococci, 353 (51.8%) of the fetuses, and actinobacilli, 98 (14.4%) of the fetuses. Eight of these fetuses yielded both bacteria. Tissues from 32 (4.7%) fetuses were overgrown by saprophytic microorganisms within 24 hours of incubation, which would mask the growth of significant bacteria. No significant bacteria were isolated from the tissues of 140 (20.5%) of the 682 fetuses.

Leptospires were not seen by FAT in any of the examined fetal tissues. Bacteria were seen by dark-field microscopy in the stomach content of many of the fetuses. The morphology of the bacteria seen correlated directly with the morphology of bacteria that were isolated from the fetuses.

Four (4 in 2001 and 0 in 2002) of the 682 fetuses were positive by FAT for EHV-1. In 2002, all 249 fetuses were negative by FAT for EVA; none were tested in 2001. Except for the four FAT-positive EHV-1 cases, no evidence of viral infection was observed in the cell cultures set up from the fetuses at the UKLDDC. From the 10 cases sent to the NVSL, there was no evidence of viral infection in cell cultures, eggs, or mice.

Table 1. Bacteriological findings on fetuses received in 2001 (433 fetuses) and 2002 (249 fetuses).

	Number (%) of Fetuses		
Bacterium Isolated	**2001**	**2002**	**Total**
Non-beta-hemolytic streptococci	223 (51.5)	112 (49.0)	245 (50.6)
Actinobacilli	74 (17.1)	16 (6.4)	90(13.2)
Actinobacilli & non-beta-hemolytic streptococci	8 (1.8)	0	8 (1.2)
Escherichia coli	7 (1.6)	8 (3.2)	15 (2.2)
Pantoea agglomerans	4 (0.9)	6 (2.4)	10 (1.5)
Serratia marcescens	2 (0.5)	4 (1.6)	6 (0.9)
Aeromonas species	4 (0.9)	2 (0.8)	6 (0.9)
Enterobacter species	0	5 (2.0)	5 (0.7)
Acinetobacter species	4 (0.9)	0	4 (0.6)
Beta-hemolytic streptococci	2 (0.5)	2 (0.8)	4 (0.6)
Staphylococcus species	1 (0.2)	3 (1.2)	4 (0.6)
Other coliforms	4 (0.9)	2 (0.8)	6 (0.9)
Other bacteria	4 (0.9)	3 (1.2)	7 (1.0)
No significant bacteria	70 (16.6)	70 (28.1)	140 (20.5)
Overgrown by saprophytes	26 (6.2)	6 (2.4)	32 (4.7)
TOTALS:	433 (100)	249 (100)	682 (100)

Discussion

Since there was no single or combination of tests that definitively diagnosed all cases of MRLS-related losses, the data in this study were from all the fetuses received during the two MRLS outbreaks. The common infectious causes of equine abortions (6,7), such as EHV-1, *S. zooepidemicus*, *E. coli*, and *Leptospira* spp., were recovered from only a few (<9.0%) of the fetuses received. The fetuses with these infections probably represented the normal fetal loss that would occur without the presence of MRLS. Most of the fetuses were infected with non-beta-hemolytic streptococci or actinobacilli. These bacteria have not often been associated with equine abortions. During a six-year study of 3,527 fetuses submitted to the UKLDDC, non-beta-hemolytic streptococci were isolated from only 26 fetuses and actinobacilli from 14 fetuses (6). A review of the records at the UKLDDC for the equine fetuses received between April 26 and May 31 in 1999 and 2000 revealed that only three were infected with actinobacilli and 11 with non-beta-hemolytic streptococci (Table 2). However, these bacteria were isolated from 433 (66%) of the 682 fetuses cultured during the MRLS epidemics. For the time period involved, the fetal loss was about 75 fetuses per year in 1999 and 2000 (Table 2), the two years preceding MRLS. If this number (75 per year) is used as normal fetal loss, then the actual number of fetuses that died due to MRLS for the two years was 532 (682 minus 150). Therefore, about 83% (443 of 532) of the fetuses from MRLS-related losses were infected with non-beta-hemolytic streptococci or actinobacilli. Also, bacteria with the morphology of streptococci or actinobacilli were seen by histopathological examination in the tissues of more than 80% of the LFL associated with MRLS (1).

Presently, only streptococci from 12 of the fetuses have been speciated, and all were *Streptococcus bovis*. Most (277) of the other streptococcal isolates were biochemically very reactive and similar, but not identical, to *Streptococcus mutans* (8). Studies are under way to identify these isolates using phenotypic and genotypic characteristics.

The species of *Actinobacillus* isolated from the fetuses remains to be determined. Recently, the classification of actinobacilli from horses was examined using phenotypic characterization and DNA-DNA hybridization (9,10). The species reported from horses were *A. equuli* subsp. *equuli* subsp. nov., *A. equuli* subsp. *haemolyticus* subsp. nov., *A. arthritidis*, Bisgaard taxon, and *Actinobacillus* genomospecies 1 and 2 (10,11). Studies are in progress to determine if the actinobacilli recovered from the fetuses associated with MRLS are identical to the species of *Actinobacillus* listed previously.

Conclusions

Based on the results obtained from the microbiological analysis of LFL submitted to the UKLDDC during the MRLS epidemics, the following conclusions were made. Viral infections, such as EHV-1 and EVA, that can be detected

Table 2. Isolation of non-beta-hemolytic streptococci and actinobacilli from fetuses: April 26-May 31, 1999-2002.

	Year			
Number of Fetuses	**1999**	**2000**	**2001**	**2002**
With streptococci	2	9	231*	112
With actinobacilli	0	3	82*	16
Without streptococci or actinobacilli	71	78	128	111

* Eight fetuses were infected with both non-beta-hemolytic streptococci and actinobacilli.

using common animal virological procedures (FAT, tissue culture, egg inoculation, and suckling mouse inoculation) were not involved in the MRLS epidemics. Bacteria such as *S. zooepidemicus*, *E. coli*, and *Leptospira* spp. that are common agents of equine abortions were not responsible for the MRLS epidemics. Non-beta-hemolytic streptococci or actinobacilli, two groups of bacteria not normally considered to be important causes of equine abortions, were recovered from most of the fetuses associated with MRLS. Their role in MRLS is unknown, but most of the pathological lesions observed could be attributed to infection by these bacteria.

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An Overview of Reproductive System Changes during and after Mare Reproductive Loss Syndrome

W. W. Zent

MARE REPRODUCTIVE LOSS SYNDROME (MRLS) CAUSED abortion in early pregnancy, and even though the abortion happened with two months remaining in the breeding season, only rarely were these mares able to get back in foal. Mares that aborted with MRLS often were contaminated with bacteria immediately after they aborted. The most common organisms isolated were non-beta hemolytic streptococcus and actinobacilli; however, in almost all instances, these organisms were quickly cleared from the reproductive tract, and the mare was free of the bacteria. The primary reason for these mares not becoming pregnant was their refusal to cycle normally, due to the presence of eCG (equine chorionic gonadotrophin) in their circulation. The eCG levels are maintained due to the presence of endometrial cups produced by the placenta before the pregnancy was lost.

Endometrial cups form between 34 and 38 days of gestation from specialized trophoblastic cells called the chorionic girdle. The chorionic girdle forms between the allantois and the yoke sac and begins to invade the uterine wall at about 30 days of gestation. Once invasion of the uterus is complete, the girdle cells lose their attachment to the trophoblast and become part of the uterine wall. They mature at about day 60 and slough between days 100 and 110. The endometrial cups derive their nourishment from the uterus, not the placenta, so that they will continue to function and produce eCG even in the absence of the placenta and fetus.

The secretion of eCG is first detectable at about day 40 and peaks at day 60. It is detectable in the circulation until days 120 to 150. The presence of eCG in the mare's circulation even after the sloughing of the endometrial cups is due to its long half-life (6 days) and its high levels in mare's plasma. It has luteinizing and follicle-stimulating activity and in non-equine species can be used as a follicle-stimulating drug. In the equine, its role is as a luteinizing agent. Secondary follicular development during early pregnancy is stimulated by follicle-stimulating hormone (FSH), which is secreted from the pituitary gland. There is evidence that this occurs in 10- to 11-day cycles and continues until mid-gestation. This FSH stimulation causes secondary follicular development, and these follicles are driven to luteinize by the circulating eCG. The corpora lutea formed by these follicles provide a secondary source of progesterone that lasts until mid-gestation when the placenta takes over progestin production.

The above process works well unless the fetus dies and an attempt is made to encourage the mare to return to estrus so that she can be rebred. Because of the presence of corpora lutea in varying stages of maturity and follicles that are also of different ages, the administration of a single injection of prostaglandin rarely will destroy all of the luteal tissue and allow the mare to return to estrus.

Attempts to rebred mares that have lost fetuses from any reason after the formation of endometrial cups have in general not been very successful. The occasional mare may become pregnant, but this is usually, in the author's opinion, due more to luck than skill. It is also important to be sure that the mare had functioning endometrial cups, with positive eCG blood levels. Some fetuses, due to an accident in development, will not form cups or at least the mare will not have circulating eCG, and these mares will usually return to cycling with very little intervention.

Several therapy regimens have been tried to encourage mares with endometrial cups to return to estrus. Multiple doses of prostaglandin will eventually destroy all of the luteal tissue, and if the mare has a functional follicle, they will return to estrus. Due to the presence of eCG, these mares have a tendency to very quickly luteinize these follicles and go out of heat without ovulating. Mares have been treated with progesterone and estradiol in an attempt to inhibit the pituitary from producing FSH and allow the ovaries to become more synchronized. This method allows all the corpora lutea to become prostaglandin sensitive at the same time and reduce the need for multiple doses of prostaglandin. This has met with some success, but the problem of eCG causing follicles to luteinize before ovulation still remains. Mares that lose their fetuses at a later stage in gestation, 80 days or more, are likely to have ovaries that become quiescent, and even though the corpora lutea are destroyed, these mares tend not to produce follicles. Their ovaries remain inactive even following attempts at stimulation. Products such as natural gonadotropin-releasing hormone (GnRH), deslorelin, sulpiride, and domperidone have all been tried with very little documented success.

Most mares with functional endometrial cups that have lost fetuses will remain in this cyclic state until about 150

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days post-conception, at which time many will return to normal cyclic behavior. Several of the mares that suffered from MRLS during the 2001 northern hemisphere breeding season were bred to the southern hemisphere season and readily became pregnant. The mares that lost pregnancies to MRLS in 2001 behaved normally at the start of the 2002 breeding season.

Suggested Reading

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Summary

K. Benirschke

THANK YOU FIRST OF ALL FOR INVITING ME TO COME TO Kentucky again. I was here many years ago, and I enjoyed it then and am enjoying it now very much.

I'm supposed to make some sensible remarks, and they may not be sensible, but they're out of the box. What this problem reminds me of very much is that in the 1960s we had the same hand-wringing in human pathology—I'm a human pathologist—about the occurrence of amelia, or absence of arms, fingers, and feet in humans. It was during the thalidomide epidemic, if you all recall, that started in Germany from a drug that was thought to be absolutely harmless, Contergan. In tests in animals, the drug was harmless. This is the reason I have totally different ideas about this process. Lenz, a German pediatrician and the son of a geneticist, visited patients with flipper arms in Hamburg, Hannover, and Cologne. Such cases had never been reported previously, but he concluded that the cause was of environmental origin.

How does all this fit together? This epidemic is just like the flipper arms, which in one significant experiment in a pregnant rhesus monkey was shown to be due to a single exposure on a specific day. One pill caused flipper arms in a rhesus monkey. You could poison rabbits and rats with Contergan, and it caused no problems, except in one rhesus monkey. So can I—with an interest in the human placenta—add anything to your discussion of the fetal loss syndrome that you experience in mares?

Placentation is very different in the mare from that of primates. Ascending infection of the placenta is the most important disease that obstetricians in human biology have to contend with. It is the principal cause of cerebral palsy and of premature birth. We have eliminated hyaline membrane disease and many other diseases by prenatal or neonatal care. Many babies born prematurely at 20 to 25 weeks that develop brain hemorrhages are the result of chorioamnionitis because of infection *in utero*. They aspirate *in utero* and swallow infected amnionic fluid containing organisms including ureaplasma and Chlamydia and other agents that enter via the cervix into the amnionic cavity. One exception as a result of maternal septicemia is *Listeria monocytogenes,* which causes abscesses in the placenta. I cannot believe the bacteria you find in the foals can get there any other way than by ascending infection. You've got to find out how that comes about.

I have had the privilege of seeing sections that Dr. Sebastian sent me of cases, and there are some significant differences from the pathology we see in human placental chorioamnionitis. One is that you see leukocytes on the umbilical cord surface, but you hardly ever see any in the umbilical vessels themselves. In humans, we see a large number of vessels packed with leukocytes. In the equine fetuses, aspirated bacteria are present in the stomach and the lungs, but that is secondary. They entered, I assume, through the cervical star, and I couldn't agree with you more that you have to examine the cervical star much more carefully for bacteria.

Even though it is often hypothesized by obstetricians that it is a prolonged rupture of the membranes in humans that causes the chorioamnionitis, in very many cases there is no rupture of the membranes, and bacteria just penetrate the membranes and invade the amnionic sac.

Human obstetricians refer to abruptio placentae cases in prematurely delivered babies with amnionitis and chorioamnionitis. Abruptio is detachment of the placenta from the uterine wall. In these cases, it is a separation at the insertion site of the membranes at the lower pole of the uterus. It is not truly a retro-placental hemorrhage. From what I've seen of the placentas that Dr. Sebastian has sent me, I do not think that the "red bag" syndrome is the cause of fetal death, nor of the problem. I think it's the end result of a very expulsive phenomenon.

We see a lot of intrauterine chorioamnionitis in humans, for many different organism reasons, including streptococci infection from which babies rarely die *in utero*. It is uncommon early in pregnancy; it's usually halfway

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through pregnancy. The babies are born prematurely, they may have sepsis and are taken care of by neonatalogists, but they don't die *in utero*. It seems to me that many of the foals have died *in utero* sometime earlier, and we need to find out if it's a toxin and what kind of toxin it is.

Why do babies die *in utero*? It reminds me of an old experience. Babies die *in utero* often when their mothers are diabetic. When the sick foals are born, they're hypoglycemic, in shock, and cold. This is also true of diabetic mothers' newborns—they are immediately, severely hypoglycemic. In order to prevent neonatal death of diabetic mothers in pregnancy and delivery, doctors in Boston years ago performed Caesarean sections so the babies wouldn't die in the last few weeks of pregnancy. Most of the placentas of diabetic mothers that I now see are induced or Caesarean sections a week and a half or so prior to their expected date of delivery because of the fear of intrauterine death. I believe there is some good correlative evidence for this in the foals—I'm thinking out of the box—that this is due to hypoglycemia *in utero*. The foal doesn't have enough to eat, so to speak, because changes in the pancreas reduce the blood sugar.

A toxin may cause the demise of glucagon-producing cells in the pancreas resulting in death of the foal or fetus. There may be subtle toxins as in the thalidomide cases. The children were perfectly normal; they just had significant reductions in the growth of the extremities. The effects may be on one organ system like the islets of Langerhans. I say this because we see a lot of newborns with islets that show beta-cell hyperplasia because of the tremendous increase in obesity.

Session 3

Epidemiological and Climatic Correlates of Mare Reproductive Loss Syndrome

Chairperson: Dr. Atwood C. Asbury, The Grayson-Jockey Club Research Foundation, Lexington, Kentucky

Epidemiological Correlates of the 2001 and 2002 Episodes of Mare Reproductive Loss Syndrome

R. M. Dwyer

FOLLOWING AN EXPLOSIVE OUTBREAK OF EARLY FETAL LOSS (EFL) and late fetal loss (LFL) in late April through May 2001, extensive investigation of mare and fetal samples and tissues revealed no definitive cause. Field investigations into mycotoxins, fungal endophytes, ergot alkaloids, and other forage factors were also negative. An intensive field epidemiological survey was undertaken to determine factors associated with Mare Reproductive Loss Syndrome (MRLS).

Significant factors associated with EFL were presence of moderate to high concentrations of eastern tent caterpillars (ETC) in areas where mares grazed, farm population of greater than 50 mares, presence of barren or maiden mares in affected fields, mares bred in February of 2001, presence of wild cherry trees in or around mare pastures, and the frequent presence of waterfowl on the farm (1). The only protective factor found was feeding hay to mares outside.

Observations made on farms by private and University of Kentucky agronomists detected a correlation between cherry trees infested with ETC and fields with MRLS-affected mares. Based on these preliminary diagnostic, epidemiological, and observational findings, a contingency plan was formulated for 2002. The plan emphasized minimizing exposure of pregnant mares to ETC and cherry trees. The following study was performed to determine the extent of ETC control measures and EFL losses on farms compared to 2001.

Materials and Methods

A questionnaire was designed to determine the numbers of 2002 EFL on farms as of June 1, 2002. These data could not be determined from Jockey Club records until 2003, and an estimate was needed by the equine industry. From 2001 MRLS survey experience, the fastest and most accurate way to disseminate a questionnaire was through the Kentucky Thoroughbred Farm Managers fax system. In 2001, an initial questionnaire by this method had a 59% return rate with a 72-hour turnaround time (2).

The questionnaire involved questions about methods of ETC control measures; whether MRLS losses were on pastures at the periphery or central part of the farm; and, if pastures were on the periphery of the farm, whether they abutted urban areas, railroads, or non-equine premises. The one-page questionnaire was faxed to 263 farm managers on June 3, 2002, requesting a return date of June 7, 2002. Data were entered on database software for analysis (Microsoft Excel 97, Microsoft Corporation, Redmond, WA).

Results

Early Fetal Loss

Of 263 questionnaires, 92 (35.0%) were returned by farm managers in 10 counties. Of 2,277 mares bred between February 1 and April 1, 2002, replies indicated that 2,102 (92.3%) mares were still in foal as of June 1.

Farm mare populations ranged in size from two to 300 mares bred as of June 1, 2002, with an average of 53 broodmares. Responding farms indicated that in 2001, 75 had EFL, 14 had no EFL, and three did not answer. For 2002, 33 farms had EFL consistent with MRLS, 55 had no EFL, and four did not answer. Of the 33 farms with EFL in 2002, 22 (67%) had lower losses than 2001, seven had an equal number of losses, three had higher losses, and one did not answer.

ETC Control Measures

The types of caterpillar control measures used on the farm during 2002 are described in Table 1.

The majority of farms implemented between two and five control measures, as shown in Table 2.

A comparison was made between the number of measures used by farms having EFL in 2001 and 2002 versus farms having EFL in 2001 but not 2002. Farms having EFL in both years used an average of 3.1 control measures; farms not having EFL in 2002 used an average of 3.4 control measures. No individual technique was identified that

Table 1. Measures taken by farm managers to decrease exposure of mares to ETC in 2002.

	Respondents	
Spraying trees with pesticides	66	(71.7%)
Keeping mares stalled during frost warnings	59	(64.1%)
Keeping mares stalled at night	49	(53.3%)
Eliminating cherry or crab apple trees	38	(41.3%)
Limiting mare's pasture exposure (daytime)	37	(40.2%)
Manual removal of ETC eggs or tents	25	(27.2%)
Tree injection to make leaves toxic to ETC	5	(5.4%)

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made a significant difference between whether farms had EFL in 2002 or not.

Location of EFL Mares on Farms

For the 33 farms that had EFL in 2002, 25 (78.1%) had losses in mares housed primarily on the periphery of the farm, whereas only two had losses in centrally located paddocks, and five farms had losses in both areas (one survey had no response).

Of the 25 farm managers reporting losses primarily from mares on the periphery of the farm, 15 (60%) reported these fields were adjacent to non-equine premises, subdivisions, or railroads.

Discussion

The study was undertaken at the request of the Central Kentucky equine industry to determine 2002 EFL losses and the types of measures used to keep mares out of proximity of ETC. Inherent bias in mailed or faxed surveys always exists, and the above data must be interpreted with participant self-selection bias in mind.

The 92.3% pregnancy rate from these farms correlates well with the study performed by local equine clinics that reported a 90% pregnancy rate of 3,089 mares examined by ultrasound (3). The computerization of Thoroughbred breeding records on Kentucky farms greatly assisted in the compilation of these figures.

Extensive efforts were made by farms to control exposure of mares to ETC, and each measure involved extensive labor and added expense. Some farms that eliminated all wild cherry trees had no MRLS at all in 2002, after suffering extensive losses in 2001.

With only 33 farms having EFL in 2002 and 25 having losses in mares pastured on the periphery of the farm, further field investigations are needed to document the locale of the pastures versus size of farm, presence of cherry trees, and the types of adjoining property in order to make any significant conclusions.

When comparing similarities between the 2001 and 2002 foaling seasons, several points are obvious in respect to MRLS. EFL, LFL, pericarditis, and endophthalmitis cases all began in late April/early May of each year; no primary infectious etiologies were detected in animal samples; no toxins, endophytes, ergot alkaloids, mycotoxins, nitrates, nitrites, etc., were detected in pasture samples. Pastures with concentrations of ETC were correlated with EFL and/or LFL.

In contrast, the differences between the two years showed a dramatic decrease in the numbers of EFL, LFL, pericarditis, and endophthalmitis. The weather was milder in 2002. The use of pesticides varied from 0 of 133 survey farms in 2001 to 89 of 92 farms using one or more ETC control measures in 2002, with 71.7% utilizing pesticides.

Table 2. Numbers of ETC control measures used by farms in 2002.

Number of Measures	Number of Farms
1	12
2	18
3	21
4	18
5	18
6	1
7	0
no answer	3
other	1*

* Kept mares out of pastures with ETC.

Extensive efforts have been undertaken to determine the cause of MRLS, but identifying the etiology(ies) of a new disease is complex. With infectious agents, fulfilling Koch's postulates is critical. With diseases of environmental, toxic, noninfectious, or multifactorial nature, Sir Bradford Hill's Causation Criteria have to be fulfilled (4). They include the strength, consistency, and specificity of the association, temporal relationship (exposure must precede the disease), biological gradient (more exposure = more disease), biologic plausibility, coherence (proposed etiology should not conflict with factual information about the disease), evidence from experimentation, and analogy. While meeting all the criteria is not necessary to "prove" causation, the first three factors are considered the most important and need to be kept in mind as an investigation progresses.

Conclusion

Based on information from 92 Central Kentucky Thoroughbred farms, the overall EFL rate was lower in 2002, with fewer farms having EFL losses in 2002 than in 2001. Multiple control measures were undertaken to limit exposure of broodmares to ETC on the majority of farms. Observed similarities and differences in diagnostic results, weather data, temporal relationships, and the continuing ETC factor indicate that the study of ETC in the role of MRLS is critical.

Acknowledgments

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Mare Reproductive Loss Syndrome in Southeastern Ohio, Spring 2001

G. S. Frazer

THE FIRST REPORT FROM A VETERINARIAN ABOUT AN UNUSUAL number of late fetal losses (LFL) in southeastern Ohio was received during the first week of May of 2001. A telephone survey of 37 veterinarians who perform a significant amount of broodmare work in Ohio localized the problem to the Appalachian foothills in southeastern Ohio (15 veterinarians). The LFL cases occurred south of a diagonal line from Cincinnati to Wheeling, West Virginia. Much of this region is comprised of heavily wooded terrain designated as the Wayne National Forest. The region contains a high number of black cherry trees, and a heavy infestation of eastern tent caterpillars (ETC) occurred during late April and May of 2001. One veterinarian from southeastern Ohio reported on numerous cases from several counties in West Virginia that are contiguous with the Ohio River.

There were no reports of similar fetal losses or ETC infestations from veterinarians in southwestern (five), central (eight), northeastern (seven), or northwestern (two) Ohio. A large farm that foaled 285 mares and bred more than 500 mares in 2001 did not report an increased incidence of either LFL or early fetal loss (EFL). This operation is located east of I-70, between Cincinnati and Columbus, in a region that is characterized by flat cropping country and minimal wooded areas. There were no reports of ETC from that location. County Extension agents did not report any unusual reproductive problems in either beef or dairy cattle herds in southeastern Ohio.

The epidemiologic investigation was impeded by the nature of the horse industry in this part of the state. Most owners raise horses as a hobby and do not rely on them as a significant source of income. Unlike the situation in Kentucky, the state veterinary diagnostic laboratory (VDL) charged more than $100 for a necropsy on an aborted foal. These economic factors meant that few fetuses were submitted for examination. Veterinarians were asked to note numbers reported in their practice, and 165 LFL cases were documented by the end of May of 2001. The majority of the abortions occurred in mares that were within four weeks of their expected foaling date. Mares aborting close to term were not agalactic and usually experienced premature placental separation or "red bag" syndrome. Some fetal membranes were described as being edematous. Most fetuses were stillborn. Some weak foals survived for a short time but succumbed to respiratory distress. Three veterinarians reported a total of 14 cases of EFL. A total of five cases of severe unilateral uveitis (endophthalmitis syndrome) was reported in foals. These neonates had corneal edema and hyphema (hemorrhage into the anterior chamber).

Based on reports from Kentucky, the Ohio VDL determined that six aborted foals exhibited lesions that were consistent with MRLS. At least three cases had prominent fibrinopurulent omphalitis (funisitis) and amnionitis. The umbilical cord lesions were limited to the amniotic segment. Bacterial isolates from aborted foals included heavy growths of *Enterobacter amigens*, *Bacillus* spp., *Serratia proteamaculans*, *Streptococcus bovis*, and moderate *E. coli*. Three horses with effusive, fibrinous pericarditis were presented for necropsy at the state VDL, and two further cases were admitted for treatment in the veterinary hospital at the Ohio State University. The pericardial sac was distended with fluid. Thick mats of fibrin diffusely coated the visceral layer of the pericardial sac and also diffusely covered the epicardial surfaces of the heart. A heavy growth of *Actinobacillus equuli* was isolated from one case.

Permission was obtained from Dr. Roberta Dwyer to use the format that was developed for the large epidemiologic survey in Kentucky. Veterinarians from southeastern Ohio

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provided contact information for those clients who were most likely to participate. Many of these subsequently declined, but 18 owners with the most reported LFL cases (117) did complete the survey. One farm reported 45 abortions within a one-week period and had experienced an especially heavy ETC infestation. Breeds represented in the LFL numbers included Thoroughbred, Standardbred, Quarterhorse, Tennessee Walking Horse, Arabian, paint, Pony, Miniature, Appaloosa, Belgian, and Percheron. The most consistent finding from the survey was the answer to the question about the severity of ETC infestation. The southeastern Ohio respondents were unanimous in that blankets of ETC covered trees, pastures, fences, stalls, hay, and water supplies. Most of the owners fed their mares on the ground, and the horses were predominately at pasture both day and night.

A follow-up survey was conducted in the spring of 2002. The majority of respondents noted that ETC were not an issue this season or that they were seen in limited numbers. Reports of an occasional abortion in southeastern Ohio were consistent with historical data. However, two cases of funisitis in aborted fetuses and one case of fibrinous pericarditis were reported from southeastern Ohio in the spring of 2002.

The conclusion from this study is that there is a circumstantial link between the heavy ETC infestation in southeastern Ohio during the spring of 2001 and the high number of LFL cases—plus unilateral uveitis and pericarditis cases—that occurred concurrently. The limited number of LFL reported in the spring of 2002 is consistent with the minimal number of ETC noted in southeastern Ohio.

Climatic Correlations of the 2001 and 2002 Episodes of Mare Reproductive Loss Syndrome

T. Priddy, C. Pieper, and W. Wang

SINCE MAY OF 2001, THE AGRICULTURAL WEATHER CENTER (AWC) has completed numerous weather analyses to determine if there were weather and climate correlations with Mare Reproductive Loss Syndrome (MRLS). According to the MRLS Web site (<www.ca.uky.edu> or <www.uky.edu.Agriculture/VetScience/gluck1.htm>), 2002 had many more equine abortions than did 2001, with 823 equine abortions during 2002 and 1,024 during the same time period in 2001. During this same time period from 1996 to 2001, an annual mean of nearly 640 equine abortions had been submitted for examination. The lack of the number of years with the occurrence of MRLS presented significant problems, but at the minimum, weather comparisons could be made between years where MRLS had occurred. In retrospect, there is evidence that cases of MRLS occurred in 1981 (1). The ultimate objective was to determine weather and climate predictors that could be monitored so that future advisories can be issued by the College of Agriculture if weather patterns begin to mirror past weather conditions when MRLS had occurred. Weather-related risk management factors were determined to be the rate of change of temperatures in the form of degree days (base 50°F) during the spring, the occurrence and frequency of frost, and the accumulation of heat as determined by analyzing threshold temperature data.

Materials and Methods

Weather and climate data were utilized from AWC databases, National Climate Data Center (NCDC), and the Midwest Climate Center, which is a regional office of NCDC. Weather and climate data from Kentucky weather stations included first-order National Weather Service weather stations and Kentucky Climate Cooperative weather/climate stations. Comparisons and analyses were conducted for maximum and minimum temperature and departure from normal, degree days (base 50°F), temperature variation, precipitation and departure from normal, soil temperature, dewpoint, and cloud cover. The complete analysis is available at: <wwwagwx.ca.uky.edu/MRLS.html>.

The focus of the study was Lexington, Kentucky, with data available from Bluegrass Airport, which is five miles west of the city, and Spindletop Research weather station, which is seven miles northwest of the city. In addition, regional climate data were utilized back to 1895 and are available on AWC's Web site at: <wwwagwx.ca.uky.edu/climdata.html>.

Results

Precipitation Analysis

Departure from normal monthly rainfall can be seen in Figure 1. An effort was made to find any similarities between rainfall patterns, especially during the spring months (shaded area). For example, in 2001, two of the three months during March through June experienced below-normal rainfall and the third above-normal rainfall. For the same time

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period in 2002, one of the three months was below normal, while two of the three were above normal. These patterns for 2001 and 2002 are actually *diametrically opposed*. Total departure from normal for 2001 and 2002 was -3.45 and 3.25 inches of rainfall, respectively.

Further, regional rainfall totals for March through May were ranked for the past 108 years of data available for the Bluegrass area in Kentucky. It was found that 2001 was the *tenth driest* with 9.93 inches, and 2002 was the *ninth wettest* with 18.93 inches. Overall, although pattern differences and similarities can be drawn from these sets of monthly data, higher resolution data and/or another moisture variable, such as dewpoint temperature, may be needed to further analyze moisture patterns.

Temperature Analysis

Several methods were utilized to express temperature comparisons between the MRLS years; frequency and distribution of days with maximum and minimum above or below a certain value, departure from normal, heat units or degree days, and frost.

Frequency of Days

The number of days on which the maximum temperatures were greater than or equal to 75°F was above normal for both 2001 and 2002, as shown in Table 1. While none occurred during March of either year, nearly twice the normal number of days occurred during April of 2001 and 50% more than normal for April 2002.

Monthly Temperature Analysis

Monthly temperature departures were analyzed in order to determine what, if any, correlation there was between this scale weather pattern and the occurrence of MRLS in a given year.

Departure from normal for average monthly temperature can be seen in Figure 2. Temporal similarities in average temperature departure are limited. The last two months of the MRLS season were above normal in 2001, and the first two months were above normal in 2002. In both cases, two of the three months of the MRLS season were above normal, and one month was below normal. Total deviation from normal for the two years totaled 5°F. For those years when MRLS occurred, monthly temperatures for April were record warm periods, with 1981 ranked as the third warmest, 2001 as the fourth warmest, and 2001 as the sixteenth warmest in the past 108 years. Overall, although pattern differences and similarities can be drawn from these sets of monthly data, higher resolution data may be desired to further analyze temperature patterns.

Table 1. Frequency of days with maximum temperature greater than or equal to 75ºF in Lexington, Kentucky, March - June.

Year	March	April	May	June	Total
2001	0	13	19	26	58
2002	0	10	13	28	51
Normal*	2	7	16	27	51

* Normal is based on the years 1971-2000.

Heat Accumulation or Degree Day Analysis

An additional method was needed to quantify the explosive nature of spring temperatures to analyze the amount of heat accumulation or "degree days" that occurred. A degree day is calculated using the daily average temperature (sum the daily high and low temperatures, then divide by two) minus a threshold temperature.

Degree days are used in a number of applications such as insect growth and development (i.e., European corn borer degree days), crop growth and development (corn and rice growing degree days), and residential heating and cooling (heating and cooling degree days). The difference between each of these applications is the "threshold temperature" used to calculate the degree days. For example, one insect called alfalfa weevil develops at temperatures equal to or

Figure 1. Monthly rainfall departure from normal for January 2001 through July 2002. (Shading represents spring months.)

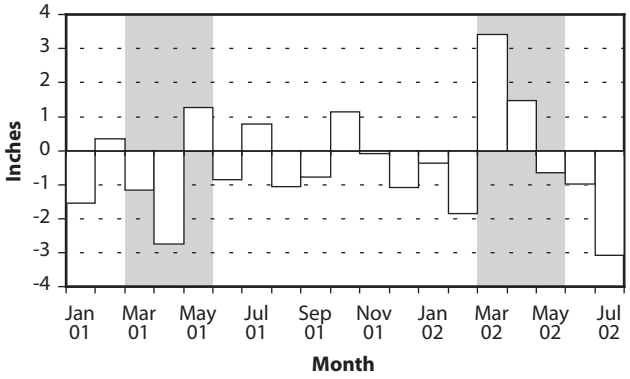
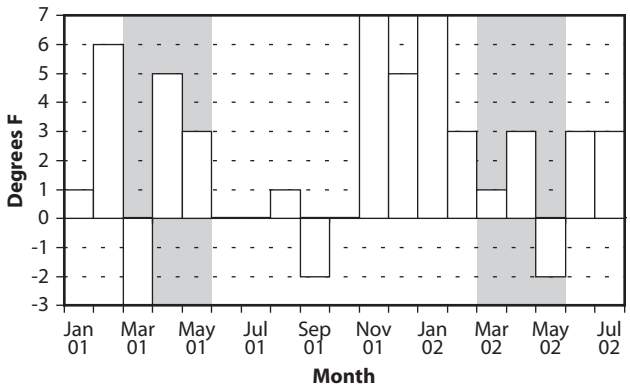


Figure 2. Departure from normal monthly temperatures for 2001 through July 2002. (Shading represents spring months.)



greater than 48°F, so the threshold temperature is 48. Corn growing degree days are calculated using a threshold temperature of 50°F, and heating and cooling degree days use a threshold temperature of 65°F.

For MRLS degree days, a threshold temperature of 50°F was selected. For a given day, if the daily high temperature was 80°F and the daily low temperature was 60°F, the following calculation provided the MRLS degree days (MRLS DDs) for the day:

$$\left[\frac{\text{(High temp + low temp)}}{2}\right] \text{ - threshold temperature = daily MRLS DDs}$$

Example: [(80˚ + 60˚)/2] - 50˚ = 20 MRLS DDs

To make these calculations, a computer program was developed that calculated the daily MRLS degree days from the daily high and low temperature for Lexington for current and past years. Additional variables calculated were the degree day accumulation, daily normal, daily departure from normal, accumulated normal, daily accumulated departure from normal, and the rate of change from normal over the previous 7 days.

MRLS degree days were plotted for 30 years. Those years in which MRLS occurred showed a significant rate of change of heat accumulation over a relatively short time period of 7 to 10 days during March and April, as shown in Figure 3. Further, low temperatures near or below 32°F during April occurred in all MRLS years, yet were not always an occurrence during non-MRLS years.

The total accumulation of MRLS degree days during March and April for 2002 was 348; for 2001, it was 334; and for 1981, it was 360. Normally during March and April, 154 degree days are accumulated. For 2002, the number was 194; for 2001, it was 180; and for 1981, it was 206.

The integration of weather events for 2002 is plotted in Figure 4. The occurrence of the 7-day rate of change of

Figure 3. Accumulation of MRLS degree days for March - April of 1980, 1981, and 2001.

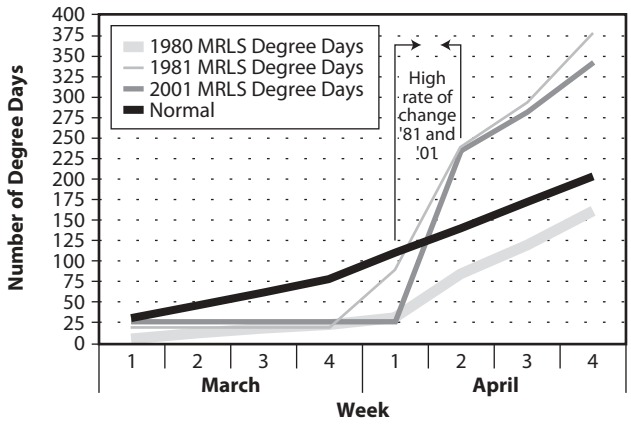
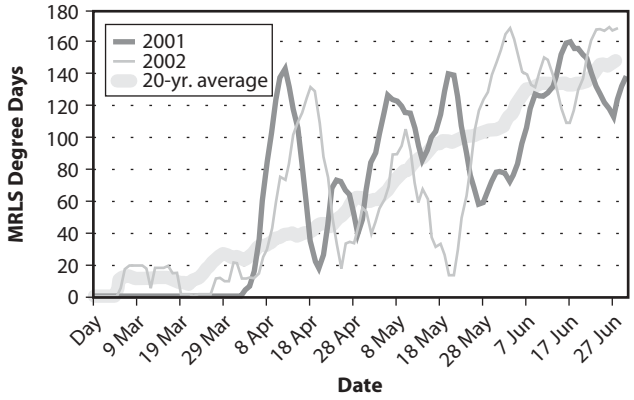


Figure 4. 7-day rate of change of MRLS degree days (2001, 2002, 20-yr. average), Lexington, Kentucky.



MRLS degree days and the total accumulations of MRLS degree days were very similar.

For March and April 2001, the maximum rate of change of MRLS degree days over a 7-day period was 143; for the same period during 2002, it was 132; during 1981, it was 96. The normal rate of change is 40 to 60 MRLS degree days during this time period.

Frost

The frequency and magnitude of minimum temperatures below 32°F are listed in Table 2 and indicate that frost was a common occurrence during the early spring season for both years. In ranking the top 20 warmest spring seasons, only one year (1985) had all of the weather-related risk management factors, including frequent frost, yet did not have MRLS reported. Prior to the year 1980, no MRLS data are available. Therefore, frost will be considered a weather-related risk management factor until more data become available.

Temperature Variation

The fact that both a quick accumulation of heat in a given season and a frost are possible risk management factors for MRLS suggests that variation in temperature may be a factor in MRLS risk management. In Table 3, it can be seen that through the 2001 and 2002 seasons, there was an above-normal number of days above 80°F and also an above-normal number of days below 32°F. Not only are these MRLS seasons abnormally warm, but they were also, in most cases, the victims of frosts and considerable day-to-day temperature variation.

Conclusions

At the beginning of the study, one very important agricultural weather variable, rainfall, was considered to be a possible predictor. Since MRLS occurred during the drought of the spring of 2001 and during the record wet spring of 2002, monthly rainfall totals and departure from normal

Table 2. Frost dates for March and April 2001 and 2002.

Year	Month	Day	Maximum Temperature (°F)	Minimum Temperature (°F)
2001	Mar	1	45	22
2001	Mar	5	36	28
2001	Mar	6	36	24
2001	Mar	7	45	28
2001	Mar	8	51	28
2001	Mar	9	42	23
2001	Mar	10	49	21
2001	Mar	18	49	29
2001	Mar	19	58	29
2001	Mar	23	63	31
2001	Mar	25	42	23
2001	Mar	26	35	19
2001	Mar	27	44	20
2001	Mar	28	55	25
2001	Apr	17	42	29
2001	Apr	18	54	28
2002	Mar	1	49	23
2002	Mar	3	27	21
2002	Mar	4	24	4
2002	Mar	5	51	26
2002	Mar	10	42	24
2002	Mar	11	52	22
2002	Mar	21	52	31
2002	Mar	22	35	16
2002	Mar	23	49	20
2002	Mar	24	67	32
2002	Mar	27	43	30
2002	Mar	28	61	29
2002	Apr	4	47	30
2002	Apr	5	50	28
2002	Apr	6	45	32
2002	Apr	7	67	32
2002	May	19	58	32

Table 3. Temperature variations for 1981, 2001, and 2002.

Days > 80	2001	2002	1981	Normal
March	0	0	1	0
April	10	7	4	3
May	11	7	6	9
June	21	24	24	21
SUM (March-June):	42	38	35	33
SUM (March-May):	21	14	11	12
Days < 32	**2001**	**2002**	**1981**	**Normal**
March	14	12	18	13
April	2	4	1	4
May	0	1	0	0
June	0	0	0	0
SUM (March-June):	16	17	19	17
SUM (March-May):	16	17	19	17
(Days > 80) + (Days < 32)	**2001**	**2002**	**1981**	**Normal**
March	14	12	19	13
April	12	11	5	7
May	11	8	6	9
June	21	24	24	21
SUM (March-June):	58	55	54	50
SUM (March-May):	37	31	30	29

rainfall was deleted as a possible weather-related risk management factor.

Several other weather-related risk management factors were determined as possible predictors of years favorable for MRLS. Heat accumulation in the form of degree days (base 50°F) was determined as a possible predictor. The rate of change of heat accumulation over a 7-day period was also determined as a possible predictor. The magnitude and frequency of frost was considered the third predictor for the occurrence of years with MRLS.

Reference

1. Bryans, J. T. Report on early fetal losses: Department of Veterinary Science, University of Kentucky. 1981.

Summary

D. Powell

I WILL CONSIDER THE THREE PRESENTATIONS AND HIGHLIGHT some aspects and conclude with one slide (Figure 1) that relates to the possible influence of population dynamics on Mare Reproductive Loss Syndrome (MRLS).

It is important to emphasize the continuing nature of the epidemiological studies. Initially, in the summer of 2001 several risk factors were identified: caterpillars and the role of cherry trees. That's exactly the purpose of an epidemiological study: not necessarily identify cause but risk factors, which enable researchers to pursue a particular path of investigation. In addition, epidemiological studies assisted in developing not only research proposals but also recommendations for what farms should do to prevent the problem in 2002. We have seen the benefits of both those initiatives.

With the experience of working in Kentucky and other parts of the world, I would like to emphasize the significance of obtaining accurate data. The equine population in Central Kentucky is an excellent resource for such information. The observations Dr. Frazer made about his

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investigations in Ohio support that statement. The Central Kentucky equine population, primarily Thoroughbred, is very well managed, with excellent records that provide good accurate data.

Another factor I'd like to emphasize in the recognition of access to data is the importance of having one diagnostic laboratory to which material is submitted from the Bluegrass area. This situation created a tremendous influx of material for detailed pathological examination. During that first week of May of 2001, material coming through the University of Kentucky Livestock Disease Diagnosis Center (UKLDDC) was overwhelming but reflective of what was occurring in the field.

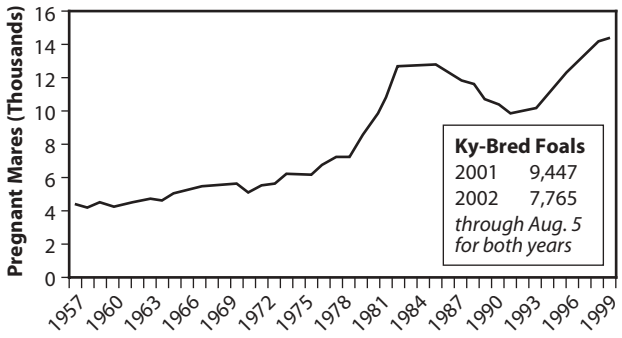
Dr. Dwyer emphasized, as a consequence of the epidemiological studies and with considerable help from the industry, farm managers, and veterinarians, we now are in a position to develop excellent baseline disease incidence data regarding fetal and foal losses allowing meaningful interpretations from year to year.

Considering Dr. Frazer's presentation, in Kentucky we learned initially through phone calls or conversations the possible distribution of MRLS outside the state. Dr. Frazer's contribution has better defined the geographical distribution. It is intriguing how it was restricted to southeastern Ohio, and the question one might ask is, "What are the factors that appear to have restricted it to that area which didn't prevail in other areas of Ohio?"

Moving to Tom Priddy's presentation, we've been working with him over the years examining weather factors that might relate to the sporadic appearance from year to year of fescue endophyte toxicosis. This culminated with the weather observations during 2001 and 2002. From the data he's presented, there's a wealth of information, and perhaps we should take a closer look at weather patterns and their influence on disease incidence.

I will conclude with a slide that examines the host-parasite relationship. A great deal of this meeting will be looking at what is the potential parasite(s) causing MRLS. This morning we examined the effect of MRLS on the mare. Figure 1 examines the host/parasite relationship at the population level looking at the annual incidence of EHV-1 abortion and how it has changed from year to year. The

Figure 1. Population of pregnant Thoroughbred mares in Kentucky 1957 - 2000.



annual Thoroughbred pregnant mare population in Kentucky is derived from the Jockey Club foal registrations each year estimated to represent 70% of the pregnant mare population of the previous year. The population changes from year to year are primarily influenced by economic forces, principally the annual sale price of yearlings. As the yearling prices increased in the early 1980s, so did the pregnant mare population, and as prices fell in the early 1990s, so did the mare population. In recent years, the population rose to the highest it's ever been. During 1981, the pregnant mare population was about 8,000. Since that time, the population has virtually doubled. A significant increase in the book size, or number of mares covered annually by each Thoroughbred stallion, has taken place. It was 40 mares per year but has gradually increased to the extent that many stallions in Central Kentucky cover more than 100 mares. In order to manage the stallion to accommodate a large number of mares over a relatively short breeding season, barren and maiden mares are mated during February and early March. It was those mares in 2001 between 40 and 100 days gestation by the end of April and early May that were exposed to the causal agent of MRLS. This provides an explanation for the large number of losses that occurred. Figures recently provided by the Jockey Club indicate the number of foals registered in Kentucky in 2001 was 9,400 and in 2002, as of August 5, it was 7,765, a 17.8% reduction.

Session 4

Potential Candidates for the Eastern Tent Caterpillar Mare Reproductive Loss Syndrome-Inducing Agent

Chairperson: Dr. Peter Timoney, Maxwell H. Gluck Equine Research Center, College of Agriculture, University of Kentucky

Placental Toxicology: Recognized Placental Toxicants in Veterinary Medicine and Consideration of a Likely Role of a Placental Toxicant in Mare Reproductive Loss Syndrome

D. H. Schlafer

AS THE PHYSICAL AND FUNCTIONAL INTERFACE BETWEEN THE conceptus and its maternal host, the placenta must fulfill many critical tasks associated with transfer and catabolism supporting fetal nutrition, portal or excretion of fetal waste, partner in the endocrine cross talk with maternal systems, and barrier to immunological rejection and invading microorganisms. Given the anatomic and functional complexities of these tissues, there is great potential for disruption by toxicants at many different points. This overview will briefly address some basic aspects of placental toxicology, discuss several toxic conditions of domestic animals recognized to have placental involvement, and examine the evidence that placental toxicity is a component of Mare Reproductive Loss Syndrome (MRLS).

Interest in the toxic effects of chemicals on placental function has been centered in two areas: drug safety testing by pharmaceutical companies and concern about human health and fetotoxicity from exposure to chemicals either through the environment or through the food supply (1-9). In regard to the latter, the most immediate concern relates to the toxic effects of cigarette smoking on the developing fetus. To a lesser degree, human placenta damage in pregnant women who abuse drugs such as cocaine and morphine is also of concern. There is strong evidence of a direct toxic effect from cigarette smoking on the human placenta (1-9). Tobacco smoking retards fetal growth, with babies of smokers being an average of 200 g lighter than normal newborns. Changes in uterine blood flow and placental development occur, and they are associated with abnormal placental vascular growth in women who smoke (1).

Pharmaceutical companies have expanded their drug safety testing programs to include more detailed study of chemicals on different reproductive processes. "Developmental toxicology" has become an important component in drug safety testing with determination of effects on reproductive cyclicity, conception rates, and embryonic and fetal losses. Second and third generations of progeny from animals dosed are also examined for reproductive performance. Until relatively recently, drug effects on placental tissues were not specifically separated from effects on the fetus, thus leaving a void in our basic understanding of both the importance of and mechanisms responsible for placental toxicity. Greater attention is now being given to placental pathology in drug testing.

Toxins and the Placenta

Toxin insult to placental function can potentially take many forms. Key biological components of placental development and function susceptible to toxic insult include altered placental development, direct cytotoxic effects on maternal and fetal placental tissue, inducers of apoptotic cell death, alternation to cell-cell adhesion, endocrine disrupters, vasoactive effects on either the maternal or fetal cardiovascular system, altered placental responsiveness to normal physiologic demands (altered homeostasis), and immune modulators and loss of immune modulation enabling maternal rejection.

Historically, the placenta was thought of as being a protective barrier, preventing passage of noxious substances to the fetus, preventing maternal rejection, and limiting any invading microorganisms from passing to infect the embryo or fetus. More recent medical evidence, however, suggests that most compounds can and do cross the placenta; some do so only at a very low rate, others more quickly. It also appears that most toxic materials that do pass through the placenta do so by simple diffusion (with the exception of some antimetabolites that are actively transported) (2).

The lipid solubility of a compound is an important characteristic with enhanced movement of chemical compounds that are more highly lipid soluble. Another important aspect of placental toxicity is that there are many enzyme systems (P450 systems, etc.) that can act on relatively inert chemical compounds resulting in substrates that are toxic ("biotransformation"). Less commonly, some compounds can directly affect placental tissues. One example of this is the production of frank placental necrosis that occurs in cadmium toxicity in rodents (10).

The toxicity of a chemical compound is dependent on the dose, susceptibility of the animal, presence of placental enzyme systems involved in either biotransformation (chemical alteration that occurs in the animal) or transport across the placenta, and stage of pregnancy. The interested reader is referred to the extensive literature on reproductive toxicology (2-9).

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Toxicities in Veterinary Medicine

There are three plant-associated toxic conditions affecting cattle, sheep, or horses that have been recognized for many years (fescue toxicity, pine needle abortion, and locoweed toxicity). Each of these is associated with pregnancy failure, and, for each, there is evidence that placental function is compromised. Fescue toxicosis in horses is caused by an endophytic fungus infection of tall fescue grasses. Cattle that ingest pine needles may abort or give birth to underdeveloped calves, and cattle and sheep that ingest locoweed (*Astragalus* and *Oxytropis* spp.) are also known to have delayed placentation, hydropys amnion, and abnormal placental development (11). The first two conditions will be discussed in greater detail.

Fescue Toxicosis

Approximately 700,000 horses in the United States are maintained on tall fescue (*Festuca arundinacea*) and therefore at risk to toxins from endophytes that grow on these grasses (12). Tall fescue is planted on approximately 35 million acres in the United States and Canada, and the direct effects on pregnancy are fairly well documented. These include a syndrome including agalactia, prolonged gestation, abortion, thickened placentas, and foal losses (12). In an early study by Garrett et al. (13), they reported relative incidence of 38% prolonged gestation, 18% abortion, and 9% thickened placentas in mares with fescue toxicosis. Fescue toxicosis is associated with an endophytic fungus infection named *Neotyphodium coenophialum* (14). Multiple toxins are produced by the endophyte, and these toxins include peramines, lolines, and ergopeptine alkaloids (12). Of these, it appears that the lolines and ergopeptine alkaloids have the most effect on the mammalian systems. Lolines have been shown to have vasoconstrictive effects on the vascular system in cattle and horses, and the ergopeptine alkaloids are agonists of the dopamine D2 receptors. The relative importance of dopamine in the reproductive aspects is to inhibit prolactin. Decreased prolactin concentrations are a major functional aspect of the pathogenesis of the agalactia and decreased mammary gland development noted in mares.

Another effect of exposure to products of these toxins from this endophyte is on the normal production by the placenta of progesterone that becomes depressed during the last 30 or 40 days of gestation. It is postulated that the reason for this is lower placental production of progesterone caused by ergot alkaloids that inhibit adrenocorticotropic hormone (ACTH) secretion in the fetus. ACTH secretion is important in controlling placental function. Progesterone normally acts in concert with prolactin and is also directly involved in mammary gland growth, development, and lactation. Interference with ACTH release is felt to be partially responsible for the failure of appropriate signaling for parturition. As noted above, prolonged gestation is a feature of fescue toxicosis.

Further effects of fescue toxicity on placental function are thought to be related to vasoconstriction (15-17). This is especially important given the very nature of the fetal and maternal placenta, of being an extensive vascular interface between them. Placental lesions that have been reported in fescue toxicity include edema, fibrosis, and mucoid degeneration of arteries (17). The incidence of retained placentas is 62% in mares consuming endophyte-positive fescue pasture grasses. This is in contrast to 12.5% for mares on fescue pastures that are endophyte negative. Clearly the toxic factors released by the endophyte have dramatic broad effects on placentation, and a study by Loch et al. (18) has shown that the fetus is also affected. Wet weights and dry fat-free weights of ponies fed different amounts of endophyte fescue seeds demonstrated that tissue dry fat-free weights increased with increasing percentages of feed in the diet. Fetal development is not only compromised, but fetal growth is altered.

Many of the clinical signs can be reversed or ameliorated by administration of domperidone, a dopamine receptor antagonist (19). A recent study by Ryan et al. (20) demonstrated that mares experimentally fed fescue contaminated with endophytes also had significant reduction in the circulating levels of relaxin.

Pine Needle Abortions

It has been recognized since the early 1950s that consumption of ponderosa pine needles by pregnant cattle may result in abortion or birth of small, weak calves and placental retention (21). Isocupressic acid isolated from ponderosa pine (*Pinus ponderosa* Laws) needles when administered to pregnant cattle induced abortions (21), and experimental studies have demonstrated that ingestion of these pine needles causes increased tone in caruncular arteries of the uterus with associated reduction in blood flow to the uterus (22).

There is little information in the literature about abortion caused by exposure to insects. Only rarely has pregnancy failure been reported to be associated with exposure to insects, and these are case reports. A pregnant woman in South America who contacted Lonomia caterpillars and went into renal failure also experienced premature labor and delivered a live baby (23). Crude extracts of cantharides are taken as part of Chinese medicine to induce abortion (24). The completion of trials to reproduce the MRLS under experimental conditions has demonstrated that ingestion of eastern tent caterpillars (ETC) leads to abortions. These studies have been critically important first steps. It will now be important to learn if there is a dose-dependent effect and, if so, if feeding of higher doses might be expected to produce more

dramatic fetal or placental lesions that could focus our attention on why and how the MRLS abortions occur.

Examination of Evidence for Placental Toxicity in MRLS

One might seek clues regarding pathogenesis of abortions in MRLS from either clinical observations or lesions found in maternal or fetal tissues. Although the syndrome is slowly becoming more precisely defined, especially now that it has been experimentally reproduced, there are still large gaps in our knowledge. Fetal losses have been reported to occur either early or late in gestation, yet given the short window of exposure and the tight breeding season, the sensitivity of the mid-gestation mare to abortions is not really known. Relatively little pathologic information is available from material from early gestation because these fetuses die so quickly and are absorbed or are autolyzed. Similarly, pathology findings reported by N. M. Williams et al. (this proceedings) are complicated by the co-existence of bacterial infection in many of the cases; more than 85% of these cases are so affected. What is the evidence that damage to the placenta is of importance in the pathogenesis of MRLS abortions?

The very high incidence of bacterial infections and the fact that the bacteria commonly isolated are not common abortifacient pathogens of the mare suggest that the placenta as a functional barrier has been damaged.

Hemorrhage and placental edema are observed in a large number of MRLS cases. The degree varies between cases, and the edema is not as severe as that found in fescue toxicity. One mechanism for the development of edema is alteration in endothelial cell permeability. This can either be the results of endothelial cell damage or cell death (apoptosis or necrosis). Severe edema can lead to placental separation. An important feature was the premature separation of the placenta ("red bag"). The pathogenesis of the placental separation has not been studied, and given the inconsistency of its association with significant edema of the chorioallantois, toxic damage to the trophoblastic cells is a consideration.

Ultrasound findings reported by T. W. Riddle (this proceedings) of echogenic allantois and to a lesser degree amniotic fluid suggests direct damage to the allantoic and amniotic epithelium and possibly the skin of these early fetuses. These changes, along with sudden fetal death, were considered the earliest and most diagnostic features of MRLS abortion in early pregnancy. Further evidence of placental involvement reflected in effects on fetal growth have been alluded to in a paper by Pantaleon et al. (to be published). Fetal femoral growth in fetuses in mares "exposed to MRLS" as measured *in utero* by ultrasound were found to be 2.5 to 7 days delayed when compared to fetuses of unexposed mares. This *in utero* growth retardation might be due, at least in part, to compromised placental function.

The rapidity of fetal demise, presence of hemorrhage, and edema suggest primary placental vascular damage in MRLS. Investigations to date have been limited by the nature of tissue available for investigation. The quality of material available for careful examination needs to be very good to detect such changes, and morphologic evidence may come only after pregnant mares are killed to enable collection of appropriate material.

More precise information about the impact of toxic compounds (from ETC) might require studies using chronically instrumented equine fetuses. Although mouse studies have begun, with some limited success, the standard model to study fetal/placental pathophysiology is the fetal lamb. Given the expense and complexity of conducting trials in pregnant mares, initiation of trials in pregnant ewes may be advised. Although the llama and pig have placentas anatomically more similar to that of the horse, there is significantly less information available about their normal fetal physiology or placental function. There is an extensive body of information available for the ovine fetus. Laboratories that use chronic instrumentation of the pregnant ewes are established in many research settings around the world. It would seem prudent to initiate either feeding or direct injection studies in pregnant sheep to determine if abortions are induced and, if so, then to plan studies using instrumented animals to define the pathogenesis of the abortion process. Disruption of the complex endocrine systems of pregnancy is also another possible pathogenetic mechanism responsible for pregnancy failure in MRLS, and chronic studies of fetuses *in utero* may be needed to characterize such an effect.

Clearly, additional animal studies should be undertaken, perhaps in those species for which there can be more in-depth evaluation of effects of fractionated compounds from ETC on the fetus and placenta.

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An Overview of Fetotoxic Agents and Their Possible Role as Agents in Mare Reproductive Loss Syndrome

R. Poppenga

THE OBJECTIVE IS TO PROVIDE A FOCUSED OVERVIEW OF reproductive and fetal toxicology, and that can be done only by painting very broad brush strokes on this subject matter. Reproductive loss due to toxicologic agents is a very complex topic because reproduction is a dynamic and complex process, and there are multiple sites that are susceptible to toxicant modification or damage. Historically, with few notable exceptions, there has been less attention paid to reproductive toxicology than to other target organ toxicology. There is a large volume of information about teratogens, but beyond these toxins that affect fetal development, relatively little is known and what is known is problematic because research must identify the multiple factors that go into a toxic effect. Here we are interested primarily in two specific reproductive toxicants, cyanide and ergopeptides, that have been investigated as possible etiologic agents in Mare Reproductive Loss Syndrome (MRLS).

Over the past several years, there has been increased interest in feto- and reproductive toxicology with concern about environmental endocrine disrupters. Much current investigation is focused on the mechanism of action of toxins that affect the endocrine system. A great amount of valuable information will probably be available in the next five to six years on this subject.

In addition to interest in the relationship between cyanide or ergopeptides and MRLS, it would be useful to examine the scientific literature for potentially toxic compounds in caterpillars. While cyanide has been shown to be present in the eastern tent caterpillar (ETC) feeding on cherry trees (1), other biologically active agents might be present that would be worth consideration. Two caterpillar genera, *Lonomia* spp. and *Lagoa* sp., have toxic substances that affect humans (2,3). However, these two genera apparently do not cause clinical problems in horses.

Reproductive toxicology is defined as the impact or potential impact of a toxicant(s), be they natural or synthetic, at any point in this process of reproduction. The impact of the toxicant can be on the sperm or the egg, the process of fertilization, implantation, embryo development, fetal development, and, after parturition, the neonate. Some effects caused by toxins are not evident until maturity. A good example of delayed effect is the exposure of expectant mothers to diethylstilbesterol (DES) in the 1950s and 1960s. Toxic effects of DES in the offspring were not observed until this population of exposed individuals (females) reached sexual maturity, about 18 to 20 years after birth.

Fetal toxicology is focused more narrowly. The reader will not find a precise definition of fetal toxicology in toxicology textbooks. However, fetal toxicology is the study of the effects of chemicals on embryos after implantation and on fetuses during development, maturation, and parturition. Fetal damage can be direct, or it can be indirect from maternal or placental effects. Direct effects require the passage of the toxicant from the gastrointestinal tract of the mother and through the placenta with subsequent distribution to target sites within the fetus. The placenta is not a significant barrier to the influx of many toxicants that could affect the fetus. The concept of the placenta as a barrier to toxicants is more related to the physical chemical aspects of the toxicant than to any true barrier that is provided by the placenta. Direct effects can be due to the parent toxicant or to a biologically active metabolite.

There are many confounding factors that need to be considered with either direct or indirect fetotoxicity, including maternal susceptibility factors such as maternal age, metabolic state, disease state, ongoing stress, nutritional state, parity, and concurrent exposure to more than one agent. Maternal susceptibility factors can lead to several important and different alterations: anemia, toxemia, endocrine imbalance, nutritional deficits, electrolyte and acid-base disturbances, decreased uterine blood flow, altered organ function, and decreased milk production. Diabetes mellitus is a problem with regard to fetal toxicity, and the physiology of the diabetic mother can have a negative impact on the fetus. Certainly conditions like stress and disease state are important parameters. Maternal hyperthermia can cause very significant teratogenic effects in offspring. Placental toxicity can lead to placental insufficiency: reduced size, reduced blood flow, altered transport, and altered metabolism.

Fetotoxicity can result from a combination of both direct and indirect effects; the ergopeptides are good examples of direct and indirect fetotoxicants. Cyanide might be an example of a direct acting fetotoxicant. However, clinical evidence is lacking.

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There are a number of compounds that are associated with abortions (4). These include plant chemicals such as isocupressic acid (from *Pinus* spp.), swainsonine (*Astragalus* spp., *Oxytropis* spp., *Swainsona* spp.), mimosine (Leucaena leucocephala) and phytoestrogens, mycotoxins such as ergot alkaloids, perloline, peramine, diacetoxyscirpenol (DAS) and ochratoxins, nitrate/nitrites, excess protein/urea, carbon monoxide, glucocorticoids, dioxins, lead, phenothiazine, prostaglandins, oxytocin, organochlorines such as dichloro-diphenyl-trichloro-ethane (DDT), and coumarin. Ingestion of several plants has been associated with abortions, but no toxicant has been identified (*Gutierrezia* spp., *Vicia villosa,* and *Juniperus* spp.).

Additionally, there are a number of other xenobiotics that are known teratogens. However, teratogenity has not been identified in association with MRLS. Therefore, known teratogens are probably not associated with the syndrome.

As mentioned, mycotoxins are associated with abortions in farm animals, and ergot peptide alkaloids of fescue prolong gestation of mares and cause birth of dysmature foals. Abortion associated with mares pastured by fescue has been reported in at least one study. Some of the other mycotoxins like DAS or ochratoxin are not commonly found in North America. It is unlikely that these mycotoxins would be the cause of MRLS in Kentucky.

A rather extensive study of causes of abortion, stillbirths, and neonatal deaths in Kentucky horses was published in the early 1990s (5). While infectious etiologies were the most common causes, a significant percentage (16%) were undiagnosed. Although it is not possible to say with certainty, some of these could be due to toxicants. In addition, other etiologic categories such as placental edema, contracted foal syndrome, and congenital abnormalities could conceivably be due to toxicant exposure.

At least one hypothesis attributes MRLS to exposure to cyanide. Certainly there is interest in this hypothesis because it has as its basis the relationship of ETC to the cyanogenic glycosides in the cherry tree and other various cyanogenic plants. The acute toxicity of cyanide/cyanogens has been investigated for a long time. There are a number of potential sources of exposure to cyanide, such as industrial processes such as electroplating, photographic processing, combustion, cigarette smoke (as a source of low-level cyanide exposure), and plant cyanogenic compounds, for example, found in *Prunus* spp. In addition, it is possible that exposure to chemicals such as acetonitrile and sodium nitroprusside can result in cyanide exposure following metabolism of the parent compound.

Cyanide is rapidly absorbed via multiple routes and is widely distributed to target organs. Cyanide readily crosses the placenta. Elimination is via first-order kinetics with variable half-lives being reported. Cyanide is highly toxic; however, it certainly is not one of the most toxic compounds. Cyanide toxicity depends on the form. For example, the LD_{50} for potassium cyanide (KCN) is 200 mg/kg body weight, which places KCN in the range of a moderately toxic compound.

Cyanide has a very specific mechanism of toxic action. It inhibits a number of enzymes, but clinical toxicosis is due to inhibition of cytochrome oxidase, which is very important for oxidative phosphorylation and energy production by cells. Electron transport is inhibited, oxygen cannot be utilized, and adenosine triphosphate (ATP) cannot be produced; so the cells that are impacted by cyanide become hypoxic. The central nervous system (CNS) is the primary target organ because the cells in the CNS are so energy-dependent. Oxidative stress and enhanced release of excitatory neurotransmitters may also contribute to CNS damage. Dopaminergic neurons in the basal ganglia are apparently the most sensitive cells in the brain and thereby are readily affected by cyanide.

Detoxification is a fairly simple process; it requires sulfur donors. The free cyanide is converted to thiocyanate by an enzyme known as rhodanese. Thiocyanate is produced and eliminated via the urine. This is a very efficient process, and free cyanide is rapidly detoxified unless exposure saturates the detoxification system. Thiocyanate is a good marker of cyanide exposure, and measurement of this metabolite may be an essential assay if further research into MRLS involves experiments with cyanide/cyanogens.

The availability of sulfur donors is a limiting factor in CNS detoxification, and it may be that the fetus has a relative deficiency in the sulfur donors compared to the mother. Therefore, the fetus might be more susceptible to cyanide intoxication. However, one critical question that needs to be asked is whether cyanide can cause fetotoxicity in the absence of toxic effects in the mother.

In looking at the literature, there has been concern about the administration of sodium nitroprusside to pregnant women for essential hypertension. Sodium nitroprusside is a good vasodilator; however, during metabolism, cyanide is released potentially causing fetal hypoxia. This concern has been evaluated using a pregnant sheep model (6,7,8,9). In these studies, the fetus was externalized and catheterized. Maternal and fetal blood cyanide levels were measured. Results suggest that fetotoxicity in the absence of maternal toxicity is highly unlikely.

This argues against cyanide or cyanogenic compounds causing equine fetal death and abortion without clinical signs exhibited by the mares.

Ergot alkaloids can be categorized as endocrine disrupters. Endocrine disrupters are exogenous agents that interfere with the production, release, transport, metabolism, binding, action, or elimination of natural hormones responsible for the maintenance of homeostasis and the regulation of developmental processes. These can be ei-

ther agonists or antagonists. Endocrine disrupters are chemically diverse and include pesticides, phytoestrogens, plasticizers, organometals, and polyaromatic hydrocarbons. Most investigations done with known endocrine disrupters have focused on estrogenic agents, androgenic agents, and compounds that affect the thyroid.

Ergot alkaloids, especially ergovaline, have been associated with reproductive effects in pregnant mares. This alkaloid, along with several other chemicals, is found in tall fescue grass *(Festuca arundinacaea*) and is produced by an endophytic fungus of the grass called *Neotyphodium coenophialum*. Ergopeptides cause both direct and indirect fetotoxicity. Indirect actions include dopamine receptor agonism, causing a decrease in circulating prolactin, and vasoconstriction resulting in placenta insufficiency. Direct actions on the fetus include inhibition of fetal adrenocorticotropic hormone secretion (ACTH) and possibly blockage of corticotrophin-releasing hormone (CRT). There is some thought that implantation of the fertilized ovum in the endometrium can be negatively impacted (10). The effects of the ergot alkaloids on equine reproduction have been studied for a number of years. The MRLS in Kentucky is quite different from that of fescue-associated impairment, suggesting that ergot alkaloids are not responsible for the syndrome.

Several "toxic" caterpillars have been identified throughout the world. Here in the United States, one caterpillar known as the "pus caterpillar" is present in Oklahoma (3). This caterpillar has venomous hairs that cause an acute local reaction, but no primary systemic effects have been reported. A species of caterpillar found in South America, the *Lonomia* species, has a real potpourri of toxic material in them that results in fairly severe chemical reactions in people and causes various coagulopathic effects (2).

There is at least one good example of how some insects utilize plant chemicals for defensive purposes. Monarch butterfly caterpillars feed on milkweed absorbing the cardioactive glycosides that are in milkweed. When birds feed on Monarch butterfly caterpillars, they become sick and regurgitate the insect. The toxic effect of the cardioactive glycoside brings about learned deterrence so that birds stop eating the caterpillars. Since ETC feed on the leaves of *Prunus* species, especially black cherry trees, these insects may contain cyanogenic glycosides. Another compound that is likely to be present in ETC is benzaldehyde. However, this compound has relatively low toxicity and would probably be unlikely to cause a problem. How ETC could cause MRLS is not defined by available information.

Because pericarditis and uveitis, perhaps due to an immunogenic stimulus, are probably components of MRLS, a brief search of cardioactive agents was done. However, none have been shown to be immunogenic. Although cardioactive agents are associated with visual abnormalities in addition to effects on the cardiovascular system, the lack of immunogenic effect would appear to rule out these types of compounds.

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A Laboratory Animal Model of Mare Reproductive Loss Syndrome: Preliminary Evaluation of a Mouse Model

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DURING 2001, CENTRAL KENTUCKY HAD AN EPIDEMIC OF early and late fetal losses (EFL/LFL) that was together called Mare Reproductive Loss Syndrome (MRLS). The LFL began in the last week of April, peaked on May 5, and declined rapidly. EFL was identified on April 26 and had a similar course and ultimately totaled about 1,500 cases (1). The same syndrome was repeated in May and June of 2002 with fewer losses. Concurrent with each epidemic was a local population explosion of eastern tent caterpillars (ETC), *Malacosoma americanum*, with large numbers wandering in pastures. Epidemiological studies, period of occurrence, and experiments conducted during the last year strongly suggest that exposure to ETC plays an important role in this syndrome.

Because of the size, long gestation period, and expense of pregnant mares, a laboratory animal was needed for study of this syndrome. It was hoped that abortions could be induced in mice by exposing them to the products of ETC. A study was undertaken to determine if ETC, frass (droppings of caterpillars), and setae (hairs) administered through different routes could produce abortion and, if so, to study the toxicopathologic effect of ETC.

Materials and Methods

Four challenge experiments with pregnant mice (ICR, Taconic Labs, Germantown, NY) were performed with ETC, frass, and setae administered by various routes. Caterpillars and frass were weighed and mixed in normal saline with a mortar and pestle. The solutions were transferred to a tissue homogenizer and finely homogenized with the volume adjusted to 0.5 ml/mouse. The homogenates were administered by gavage using a ball-tipped needle (Perfekturn, Popper & Sons Inc., New Hyde Park, NY). Setae (15 setae/mouse) were plucked from the skin of caterpillars and homogenized in a tissue homogenizer with normal saline, and the volumes were adjusted to 0.4 ml/mouse. All materials were prepared fresh daily for administration.

In Experiment 1, three groups of mice (12 days pregnant) were administered frass (19 mg; n = 9) that had been frozen, early instar ETC (70 mg; n = 9) that had been frozen, and saline (0.5 ml; n = 8) by oral gavage. The experiment was terminated on day 19 of pregnancy when the mice began to give birth. All mice and pups were euthanized, and complete necropsies were performed.

In Experiment 2, fresh frass and ETC were used. Three groups of mice (12 days pregnant) were administered fresh frass (19 mg; n = 7), late instar ETC (200 mg; n = 7), and saline (0.5 ml; n = 7) by oral gavage. The experiment was terminated on day 18 of pregnancy. All animals were euthanized, and full necropsies were performed.

In Experiment 3, two control groups of mice (5 days pregnant) were administered saline by oral gavage (0.5 ml; n = 4) and saline by intraperitoneal (IP) injection (0.4 ml; n = 4), and three treatment groups (5 days pregnant) were dosed for 14 days with one of the following: fresh frass (19 mg; n = 7) by gavage, late instar ETC (200 mg; n = 8) fed on fresh cherry tree leaves by gavage, or setae plucked from live late instar caterpillars filtered through a bacterial filter (VWR Scientific Products, West Chester, PA) by IP injection (20 setae/mouse; n = 7). The reason for injecting setae was to evaluate the possible role of a soluble setal toxin in MRLS. However, because of a personnel change occurring 4 days into this experiment, all later setal homogenate injections were unfiltered. This experiment can therefore be interpreted only in terms of a setal homogenate effect. The experiment was terminated on the nineteenth day of pregnancy. All animals were euthanized, and full necropsies were done.

In Experiment 4, three groups of mice (5 days pregnant) were treated by IP injection for 14 days. Groups were administered one of the following treatments: saline (0.4 ml; n = 7) to a control group, a filtered homogenate/extract (20 setae/mouse; n = 7), and an unfiltered homogenate/extract (20 setae/mouse; n = 7). Setae were plucked from frozen late instar caterpillars, and the homogenate was filtered through a bacterial filter. The experiment was terminated by euthanasia on the eighteenth day of pregnancy, and full necropsies were performed. Both filtered and unfiltered homogenates were injected to distinguish between a setal toxin (filtered homogenate) and mechanical irritation or bacterial-laden setae disrupting the fetal membranes (unfiltered homogenate).

Results

The gross and histopathological findings of the four experiments are detailed in Tables 1 through 4. In Experiment 1, there were no significant findings in the uteri of

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the mice that died during the experiment. Furthermore, there were no significant findings in the uteri and fetuses of any of the mice in treatment and control groups. The total number of pups born for the treatment and control group mice are shown in Table 1.

In Experiment 2, the uterus of the mouse that reabsorbed one fetus had acute suppurative inflammation at the implantation site. There were no significant findings in the uteri (implantation sites) and fetuses of any mice in the treatment and control groups. The total number of pups in the uteri of treatment and control mice are shown in Table 2.

In Experiment 3, the group administered setae had statistically significant reabsorptions of all fetuses in three mice, and two mice were not pregnant. The uteri of the mice that had reabsorptions had acute suppurative inflammation at the implantation sites. The total number of pups in the uteri of treatment and control mice and the histopathological findings in the uteri of setae-administered mice are shown in Table 3.

In Experiment 4, two fetuses were reabsorbed in the unfiltered setae group, eight fetuses were reabsorbed in the filtered group, and four fetuses were reabsorbed in the control group. The uteri of the mice that had reabsorption showed acute suppurative inflammation at the placental sites. The total number of pups in the uteri of the treatment and control group mice are shown in Table 4. The bacteria isolated from the uteri of mice in Experiment 1, 3, and 4 are detailed in Table 5.

Table 1. Number of pups for each mouse in Experiment 1. Group 1 was administered caterpillar extract (early instar), Group 2 was administered frass, and Group 3 (control) was administered normal saline.

Animal ID	No. of Pups	Comment
Grp1-1	14	DEAD - during experiment
Grp1-2	5	
Grp1-3		No pups born when euthanized - 10 at necropsy
Grp1-4	13	DEAD - during experiment
Grp1-5	11	
Grp1-6	14	
Grp1-7	12	
Grp1-8	14	
Grp1-9	12	one not active
Grp2-1	7	pups not active
Grp2-2	13	DEAD - during experiment
Grp2-3	11	
Grp2-4	12	
Grp2-5	11	DEAD - during experiment
Grp2-6		No pups born when euthanized - 11 at necropsy
Grp2-7	12	
Grp2-8	14	
Grp2-9	11	
Grp3-1	13	
Grp3-2	10	
Grp3-3	13	
Grp3-4	9	
Grp3-5	11	
Grp3-6	14	DEAD - during experiment
Grp3-7	10	
Grp3-8	12	

Discussion

In Experiment 3 (setae from live ETC), three of the five pregnant mice had reabsorption of their fetuses. The same experiment, when repeated with frozen setae (Experiment 4), did not produce reabsorption as observed in Experiment 3. The structure and composition of setae, whether it changes by freezing and subsequent thawing, are not known. Normally 10 to 20% of reabsorption is noticed in the uteri of pregnant mice. Reabsorption in the control groups of Experiments 2, 3, and 4 was not above 10%, and no significant pathological changes were observed. In Experiments 1, 2, and 4, the percentage of reabsorptions was also below 10% in the treatment groups.

It was hoped that the mouse would mimic MRLS seen in horses so that a laboratory animal model could be used to more thoroughly investigate MRLS. Horses aborted following oral dosing with 50 g ETC to a 500-kg horse (0.1 g/kg). The mice did not abort following oral dosing with 200 mg ETC to a 20-g mouse (10 g/kg). So even though mice received a 100-fold increase in ETC per body weight, they were not susceptible to abortion. The reason(s) for the difference(s) in susceptibility of the two animals could be different intestinal enzymes and/or flora, different placentation, or longer gastrointestinal tract in the horse increasing the possibility of intestinal absorption. The intestinal tract appears to be involved because, when it was bypassed with IP injections of setal homogenate/extract from live ETC, mouse reabsorptions did occur in three of the five mice.

There also appears to be a factor associated with setae from live ETC versus frozen ETC. Since Experiment 4 was run after the supply of live ETC had been exhausted, setae were taken from frozen ETC. There were no effects from IP injection of setal extract in that experiment.

Conclusion

Pregnant mice are not as susceptible to ETC-induced abortion as horses. Mice do not produce abortions where the expelled fetuses and placentas can be examined, but rather mice reabsorb the fetuses. The gross and histopathological changes in the experiments conclude that mice (*Mus musculus*) are not a suitable laboratory animal for reproducing MRLS.

Reference

1. Harrison, L. R. Kentucky equine abortion storm and related conditions. Proceedings of the United States Animal Health Association. 2001; (105): 227-229.

Table 2. Number of pups for each mouse in Experiment 2. Group 1 was administered fresh frass, Group 2 was administered fresh ETC, and Group 3 (control) was administered normal saline.

Animal ID	No. of Pups	Comment
Grp1-1	7	
Grp1-2	12	
Grp1-3	13	
Grp1-4	2	
Grp1-5	13	
Grp1-6	10	
Grp1-7	14	
Grp2-1	12	
Grp2-2	11	
Grp2-3	14	
Grp2-4	15	
Grp2-5	10	
Grp2-6	13	
Grp2-7	12	
Grp3-1	14	
Grp3-2	12	one fetus reabsorbed
Grp3-3	10	
Grp3-4	13	
Grp3-5	13	
Grp3-6	13	
Grp3-7	10	

Table 3. Pups for each mouse in the uteri of setae group of Experiment 3.*

Animal ID	Total No. of Fetuses	Reabsorption	Comment
Control 1	10		
Control 2	4		
Control 3	11	One dead fetus	
Control 4	12		
Setae 1	11		
Setae 2	11		
Setae 3		All fetuses reabsorbed	Acute suppurative inflammation at implantation site
Setae 4			Not pregnant
Setae 5			Not pregnant
Setae 6		All fetuses reabsorbed	Acute suppurative inflammation at implantation site
Setae 7		All fetuses reabsorbed	Acute suppurative inflammation at implantation site
Caterpillar group	No abnormalities noticed in the uterus and fetuses		
Frass group	No abnormalities noticed in the uterus and fetuses		
Control group-2	No abnormalities noticed in the uterus and fetuses		

* The histopathological findings of uteri of mice that had reabsorptions are listed in column 3.

Table 4. Number of pups for each mouse in Experiment 4. Group 1 received unfiltered setae, Group 2 received filtered setae, and Group 3 (control) received normal saline.

Animal ID	Pups Born	Comment
Grp1-1	1	
Grp1-2	2	Left horn thickened
Grp1-3	13	
Grp1-4	Non-pregnant	
Grp1-5	11	Normal
Grp1-6	12	One reabsorbed
Grp1-7	3 dead, 3 live	Had pups before euthanasia
Grp1-8	5 dead	Had pups before euthanasia 1 small fetus
Grp1-9	7 live, 2 dead	Had pups before euthanasia
Grp1-10	9	1 reabsorbed
Grp2-1	15	3 reabsorbed
Grp2-2	12	normal
Grp2-3	Non-pregnant	
Grp2-4	Non-pregnant	
Grp2-5	11	
Grp2-6	10	
Grp2-7	5	4 reabsorbed
Grp2-8	Non-pregnant	
Grp2-9	5 live, 2 dead	Had pups before euthanasia
Grp2-10	12	1 reabsorbed
Grp3-1	7	Had pups before euthanasia
Grp3-2	1	
Grp3-3	Non-pregnant	
Grp3-4	12	
Grp3-5	11	1 reabsorbed
Grp3-6	12	1 reabsorbed
Grp3-7	9	
Grp3-8	12	Had pups before euthanasia
Grp3-9	14	1 reabsorbed
Grp3-10	13	1 reabsorbed

Table 5. Bacteria isolated from Experiments 1, 3, and 4 and from one of the filters used to filter setae.

Experiment 1	Experiment 3	Experiment 4	Filter
No growth	*Serratia marcescens* - setae group	*Serratia marcescens*	*Pseudomonas maltophila*
	Pantoea agglomerans - setae group	*Pseudomonas maltophila*	Unclassified gram negative bacillus

Summary

Noxious Agents and Disease Patterns Relevant to Mare Reproductive Loss Syndrome

D. S. Kronfeld

THE EMPHASIS IN THIS SESSION IS ON POTENTIAL NOXIOUS agents that might cause the Mare Reproductive Loss Syndrome (MRLS). It seeks to identify the direct or proximate cause and implies a simple cause-effect relationship. This limited view of MRLS has not solved the problem in 16 months since April of 2001. In my opinion, we should be trying to describe the whole pattern of the disease complex, and my intention in this perspective is to summarize likely disease patterns and to note where agents identified by the previous speakers may fit. First we need a broader concept of cause than simply A causes B.

Causation

The cause of abortion is the set of events, conditions, and characteristics necessary and sufficient for its occurrence (1). To determine the set, the usual approach is to list all reasonable possibilities, then try to rule each out, as in differential diagnosis. Possibilities usually arise as associations, for example, MRLS with certain climatic conditions, eastern tent caterpillars (ETC), and waterfowl. Each association can be ruled out by inconsistency. Its inclusion in the causal set can be reinforced by demonstration of appropriate timing and mechanism(s).

Disease Patterns

The horse exhibits many patterns of diseases with multifactorial etiologies and multiple etiologies (2). It is unrivaled in regard to disease complexes, such as rhabdomyolysis and laminitis, in which quite different sets of causes converge on apparent constitutionally weak sites. The muscle membranes and the laminae in the foot are two such sites; perhaps the placenta is another. Multiple etiologies can give the impression of exceptions or inconsistencies and intensify caution in rule-outs.

Septic Setae

T. Tobin (this proceedings) challenges the whole idea of a toxic agent with his septic penetrating setal emboli (SPSE) hypothesis. In this disease pattern, penetrating ETC setae pave the way for oral commensal bacteria to proceed via the blood vessels to the placenta where they lodge, proliferate, and cause abortion. The bacteremia may also cause pericarditis and uveitis.

This hypothesis is novel and testable. It appeals as a disease pattern in need of further development for several reasons. ETC setae are soft compared to the spiky setae of other species that are known to damage eyes, according to T. D. Fitzgerald (this proceedings). Dental hygienists spread oral commensals to heart valves but not yet to human placentas. Penetration of bacteria is more common in the large bowel of the horse subjected to luminal acidosis.

Carbohydrate Overloads

Luminal acidosis is caused by rapid fermentation of soluble carbohydrates to lactic acid (2). The most variable fraction of carbohydrates in pastures includes fructans (fructose oligosaccharides) and soluble fibers (pectins, gums, mucins). Common pasture contents of fructans are about 5% (dry matter) but rise to 25% during rapid growth or following frost kills. Early April of 2001 was warm, which would have encouraged fructan production; then came a frost, causing fructan accumulation.

These oligosaccharides are not hydrolyzed in the small intestine but are rapidly fermented in the cecum. When pH falls below about 6.3, the integrity of the cecal epithelium begins to loosen with increasing risk of bacterial penetration. This disease pattern is best described for liver abscesses in feedlot cattle. It could also operate in the pregnant mare and enable cecal anerobes to reach the placenta. It could be an exacerbating factor in SPSE.

Acidic conditions in cecal fluids if sufficiently severe cause bacterial lysis with the release of endotoxins. These lipopolysaccharides have been implicated in one form of laminitis. They may also affect the microcirculation of the placenta and lead to abortion. Pregnant mares have exhibited brief periods of fever before abortion. An endotoxic component has not yet been ruled out for MRLS.

Overloads of hydrolyzable starch and sugar from meals of more than 5 pounds of grain-molasses sweet feeds have the same effects as fructans on cecal fermentation. Thus, nutritional management of pregnant mares during risk periods should emphasize supplements of hay but not sweet feed because overloads of carbohydrates have not been

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ruled out as conditioning factors in MRLS. If sweet feed is used, it should be given as multiple small meals (2 or 3 pounds).

Meals of hydrolyzable carbohydrates raise blood glucose concentration and initiate a feeding-fasting cycle of metabolites and hormones. Studies in progress at Virginia Tech are showing that chronic feeding of starch-and-sugar meals leads to increased insulin resistance, especially in mares during late pregnancy. Studies at the Gluck Center by Dr. B. Fitzgerald are also revealing that insulin resistance in pregnant mares is increased by obesity. A higher incidence of abortion occurs in women who have pregnancy diabetes, according to K. Benirschke (this proceedings). Until type-2 diabetes and insulin resistance are ruled out as conditioning factors in MRLS, prudence suggests the avoidance of large meals of sweet feed and obesity in pregnant mares.

Plant Poisons

Previous speakers have suggested that climatic conditions may also have promoted overgrowth and consumption of pine needles, locoweed, lupins, veratrum, and hemlock, which have been implicated as causes of abortion in cattle and horses. Only the last of these possibilities has been associated with MRLS, and it appears to have been ruled out because it was also widely available to mares on many MRLS-free pastures. Hemlock poisoning might be conditioned by another concurrent factor, however, that has not yet been identified.

Mycotoxins and Zinc

The association of MRLS with highly constrained climatic conditions suggests that a highly favorable microenvironment might favor the production and accumulation of noxious agents. Previous speakers in this session have identified fescue endophyte alkaloids and an endocrine disrupter, zearalenone. About 10 mycotoxins common in feeds have been evaluated in regard to MRLS as reported by K. Newman (this proceedings), but no broad screen likely to detect unusual mycotoxins has been reported. For example, one screening method claims to detect any of 182 mycotoxins (3).

A likely template is facial eczema, in which growth of a fungus explodes on ryegrass only during brief periods of suitable microenvironmental conditions. The fungus, *Pithomyces chartarum*, was isolated from a black line on a mower blade observed by a farm hand; it had eluded scientists working on the disease for half a century. Its toxin, sporidesmin, was similarly unsuspected. Thus, experience with facial eczema suggests that a wide net needs to be cast for any fungus and mycotoxin involved in MRLS.

Sporidesmin damages the liver, which produces a photosensitive pigment. Severity of sunburn is conditioned by marginal dietary zinc. The toxin promotes production of radical, reactive oxygen species. A key enzyme in their detoxification is copper-zinc-superoxide dismutase. If the cause of MRLS involves a similar mycotoxin, then a conditioning factor may be a high dietary copper:zinc ratio. The copper:zinc 1:4 ratio recommended by the National Research Council was challenged by a 3-fold increase in copper allowance, following studies in Ohio and Kentucky on developmental orthopedic disease. Tolerances are about 50-fold for copper but only 10-fold for zinc, so nutritionists are cautious about raising zinc to preserve a 1:4 copper:zinc ratio.

Caterpillar Consumption

The ETC has been associated with MRLS as reported by R. Dwyer (this proceedings). The first question regarding causation concerns voluntary ETC consumption by horses.

My property line in the Appalachian Mountains of southwestern Virginia has a quarter-mile stretch of cherry trees. The number of tents and ETC vary enormously from year to year. Three times in 14 years ETC have crawled everywhere, including the feed buckets of my two equids, a pony and a Percheron. ETC appear to be attracted by the residual slime of molasses, slobber, and feed dust. After a scoop of sweet feed is dumped on a few or a dozen caterpillars, the horses snuffle up the feed but sort out and leave the caterpillars. I have counted these ETC in feed buckets so often that I'm convinced—my horses do not eat caterpillars. Is that a sufficient rule-out? No, but it's a prompt for testing voluntary caterpillarphagia in Thoroughbreds.

Caterpillar Toxicity

Caterpillars could be carriers of plant toxins or be noxious in themselves; for example, their setae could be mechanically damaging, as suggested by T. T. Tobin. Four administration experiments have been alleged to demonstrate ETC toxicity, but the preliminary reports (in horse magazines and this proceedings) have provided no statistical basis for this conclusion. In fact, some of the results are overtly contradictory unless attention is given to the timeline.

On August 27, I presented the results of my application of Fisher's Exact Probability Test to the published data from these four ETC administration experiments. Subsequently, I obtained the help of Dr. Dan Ward, a statistician at Virginia Tech. He applied a generalized linear model to the data with a logit link and a binomial error distribution, with single degree of freedom contrasts used to test hypotheses of interest. In the discussion that follows, my original P values are given in plain text, Dr. Ward's in *italics*.

A convenient starting point is the June experiment of B. Bernard et al. (this proceedings) because the question was simple and the results clear. Nasogastric administration of 50 g crushed fresh ETC in a slurry with 50 ml of water resulted in EFL in 4 of 5 mares, compared to 0 of 5 mares given 50 ml water (control) or 50 ml of water containing 2.5 g of stored frass. The ETC group was different from the combined control and frass groups (P = 0.004, *P = 0.0004*), and from the frass group or the control (P = 0.048, *P = 0.004*). Thus, ETC was toxic, frass not.

The July experiment of M. Sebastian et al. (this proceedings) extended the nasogastric toxicity of fed Michigan ETC to *LFL* (P = 0.002). In addition, clinical and pathological observations indicated placental detachment leading to fetal ischemia and hypoxia. The congruence of this experimental syndrome with natural MRLS needs to be evaluated.

The April experiment of B. Webb et al. (this proceedings) used ETC fencing and permitted but did not demonstrate ETC consumption by horses. Abortion occurred in 7 of 10 mares in the ETC-plus-frass paddock, and 7 of 9 in the frass-only paddock (P = 1.0, *P = 0.70*). The combined frass groups (14 of 19) were different from the control group, which had 3 abortions in 10 mares (P = 0.046, *P = 0.021*). Clearly, the frass was noxious in April. The three abortions in the control group were most likely attributable to caterpillar/frass contamination of the control pens. The ETC body tissues were either nontoxic or not consumed in April.

In the May experiment of B. Webb et al. (this proceedings), the ETC fences were improved, and the ETC were not fed for two or three weeks, so carried little or no frass. Abortions were 1 of 8 in controls, 0 of 8 in the frass group, and 3 of 8 in the starved ETC group. The control and frass groups were not different (P = 1.0). The two statistical approaches gave different results: ETC versus frass (P = 0.20, *P = 0.027*), and ETC versus the two combined frass and control groups, that is, ETC-free groups (P = 0.091, *P = 0.033*). Clearly, frass was no longer toxic in May, but the ETC body tissues now may (logistic regression) or may not (Fisher's Exact) induce abortion.

A timeline interpretation starts with a climate-conditioned, biological noxious agent that was consumed by ETC in April and was present in the frass in sufficient amounts to cause abortion late in April and early in May. The suspected toxin started to accumulate in ETC body tissues but was not yet enough to cause abortion in April. Accumulation of the noxious agent in ETC body tissues was clearly sufficient after two or more months, that is, later in June.

These experiments would be more convincing if voluntary consumption of caterpillars was actually observed (rather than assumed) before or at the time of the spike in MRLS frequency—the last week of April and the first week of May. The timeline should also be applied to the SPSE hypothesis; soft and furry setae in April and early May are unlikely to penetrate, and hard spiky setae later in July would be less relevant to the high time of natural MRLS.

Sentinels

Association is sufficient and causation not necessary for ETC to be useful sentinels in the management of mares at risk. A profusion of tents or larvae would be an indication for reducing exposure of pregnant mares to pasture. This avoidance reduces risks associated with fructans and other plant substances, endotoxins, myriads of mycotoxins, and ETC tissues, frass, and setae—none of which have been ruled out as components in the complex causation of MRLS. The history of public health is replete with effective interventions based on associations before causation was established.

Genetics

My worst fear is that MRLS may be a side-effect of selection for speed. Has the Thoroughbred's placenta become a vulnerable site like its bleeding alveoli, leaky muscle membranes, and fragile laminae? If so, then the coincidence of several quite different environmental triggers (fructans, mycotoxins, ascending infection, and ETC, etc.) might converge on placental separation at the same time and give the appearance of an outbreak of MRLS.

A relevant disease pattern is infertility caused by a round rump and sloping vulva requiring the Caslick procedure. When I was a veterinary student 50 years ago, perhaps two or three fillies in a 100 had the colt-like oval hindquarters and needed a stitched vulva. Most fillies had a triangular rear end, with a vertical drop from the tail insert. When the anus protruded an inch during defecation, the feces fell clear. A panel of three veterinarians and three farm managers at a meeting in Lexington, Kentucky, in 1990, which I attended, agreed that over 35 years they had bred for a colt-like conformation to obtain more speed from fillies. Now, nearly all Thoroughbred mares have rounded rumps and sloping vulvas, so are Caslicked to reduce the risk of infertility associated with soiling and chronic infection.

Two testable hypotheses arise from this deliberate and overt conformational trade of reproductive unsoundeness for speed. First, could perineal conformation be a conditioning factor in MRLS? One could test for associations between sloping vulvas, ascending infections, and MRLS. Second, what if single-minded striving for speed has unintentionally endowed the racing Thoroughbred with a covert, constitutionally vulnerable placenta that is prone to separate at the slightest insult of any kind? This hypothesis could be tested retroactively by comparing the family racing performances of MRLS mares with spared mares.

Conclusion

The MRLS challenge continues. Conclusive rule-outs must be painstaking; testing for 10 common mycotoxins, for example, is hardly a comprehensive screen. Rule-outs are needed for numerous likely conditioning factors: dietary fructans, sweet feed and obesity, the copper:zinc ratio, sloping vulvas, and fast families. These factors can be evaluated retroactively by epidemiologists with the help of nutritionists, theriogenologists, and geneticists. Looking forward, the timeline is crucial in testing prospective causal agents. Are ETC setae hard and penetrating in April or July? Ethologists must watch mares through telescopes and binoculars to see if they voluntarily consume ETC while unperturbed on pastures in late April and early May. Toxicologists should compare the toxicity of frass and parts of the ETC bodies at times relating to a spike in MRLS frequency. They could also compare cyanide affinities of cytochome oxidase of tissues from mares and foals. Bacteriologists should answer two questions. Do the placental bacteria come from the mouth, cecum, or vagina? More difficult is the timeline. Are the placental bacteria primary invaders that start placental separation or later lodgers in already damaged tissues? Corrective interventions should proceed in the usual way from encouraging clinical experiences to rigorous comparative trials. In the end, we will see if MRLS represents a new pattern of disease or a variation on an old theme.

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Session 5

Mare Reproductive Loss Syndrome and Associated Syndromes: Toxicological Hypotheses

Chairperson: Dr. Neil Williams, Livestock Disease Diagnostic Center, College of Agriculture, University of Kentucky

The Potential Role of Ergot Alkaloids in Mare Reproductive Loss Syndrome

C. L. Schultz and L. P. Bush

TALL FESCUE (*FESTUCA ARUNDINACEA* SCHREB.) IS A PERENnial, cool-season grass commonly used for forage and turf purposes (1). Fescue is characteristically more drought and cold tolerant and forms denser stands than other *Festuca* species. It is also more competitive with weeds and thrives on a wider range of soil types (1). Approximately 33 million acres of tall fescue are grown in the United States, with close to 50% of that existing east of the Mississippi River. States such as Kentucky, Tennessee, Virginia, West Virginia, and North Carolina use tall fescue as their primary grass species for forage and turf production (1). Approximately 5.5 million acres are grown in Kentucky alone and are grazed by an estimated 96,000 horses and/or ponies per year (2). Despite its good nutritive value, consumption of tall fescue by livestock results in a decrease in both reproductive and growth performance. It has been found that animal performance was depressed due to an endophytic fungus (*Neotyphodium coenophialum*) present between the cells of the plant (3). Three primary classes of alkaloids produced by the fungus and that play a potential role in affecting animal performance include the ergot (ergovaline), pyrrolizidine (lolines), and pyrrolopyrazine (peramine) alkaloids.

Cattle consuming tall fescue commonly exhibit increased respiration rates and rectal temperatures; depressed intake, subsequently resulting in lower average daily gains; and a rough hair coat, which is atypical to that usually seen during the summer grazing months (4). Contrary to depressed performance observed with cattle consuming endophyte-infected fescue, horses, most often gravid mares, typically exhibit a decrease in reproductive performance. Prolonged gestation, agalactia, increased foal and mare mortality, dystocia, tough and thickened placentas, weak and dysmature foals, reduced serum progesterone and prolactin, and increased serum estradiol-17ß are all signs commonly associated with mares consuming endophyte-infected fescue (5). Arns et al. (6) fed a fescue seed-based diet containing three levels of ergovaline (0, 0.16, and 0.31 0.39 $\mu g \cdot g^{-1}$) to determine its effect on the establishment and maintenance of early pregnancy. Mares consuming diets containing ergovaline had lower serum progesterone and prolactin levels with no effect on overall conception rates, cycles per conception, or embryonic vesicle size through 28 days of pregnancy. Conversely, Brendemuehl et al. (7) found mares grazing endophyte-infected (1.2 0.39 $\mu g \cdot g^{-1}$ ergovaline) tall fescue had prolonged luteal function (22.9 versus 15.8 days) and higher embryonic death (30 versus 7.7%) than mares grazing endophyte-free tall fescue pastures. Serum progesterone and prolactin concentrations followed similar trends observed by Arns et al. (6). Although reproductive efficiency was compromised in the two previous studies, a majority of reproductive complications occur during late-term pregnancy. Putnam et al. (8) found mares grazing tall fescue from 90 days of gestation through parturition had obvious signs of dystocia (10 of 11 mares) with foal survivability greatly reduced. Mares grazing endophyte-free tall fescue produced 11 viable foals, while those grazing endophyte-infected pastures (average 0.39 $\mu g \cdot g^{-1}$ ergovaline) produced three healthy foals. Gestation was also 20 days longer for mares consuming endophyte-infected tall fescue. Ten of 11 mares grazing endophyte-infected pastures showed no evidence of udder development or lactation prior to and during parturition. Udder development was normal in all 11 mares grazing endophyte-free tall fescue.

Because research has shown endophyte-infected tall fescue pastures, containing ergovaline concentrations as low as 0.2 $\mu g \cdot g^{-1}$, negatively affect early- and late-term pregnancies, the objectives of this experiment were to observe alkaloid concentrations in horse pastures located in the Bluegrass region of Kentucky and to determine any potential role they may play in Mare Reproductive Loss Syndrome (MRLS).

Materials and Methods

Twelve area-wide horse farms and one hay farm were monitored for alkaloid concentrations during the months of March, April, May, and June of 2002. Pastures, in which clipped forage samples were collected, contained pregnant mares or mares to be bred. Four sample types were collected on farms containing cherry trees on or near the perimeter of the farm (pure tall fescue–TF, composite weed/forage mixture–COMP, inside the cherry tree drip line–IN, and outside the tree drip line–OUT). Farms containing no cherry trees only had two sample types collected (TF and COMP). Clipped forage samples for each sample type were collected from an average of six collection sites within a field on each farm. Only fields containing pregnant mares

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were sampled, and fields were sampled biweekly. Within 7 hours, and usually sooner, samples were dried in a forced air oven at 55°C for 24 hours and then ground (Wiley Mill) to pass a 0.5-mm screen. Samples were immediately analyzed by reverse-phase high performance liquid chromatography for ergovaline and ergovalinine (9). Ergot alkaloid concentrations were adjusted up 30% due to losses that occur with oven drying. Lolines (N-acetylloline [NAL], N-formylloline [NFL], and N-acetylnorloline [NANL]) were analyzed using gas chromatography.

Where appropriate, data were analyzed using the Proc GLM procedure of SAS. Due to sampling procedures and different management practices employed by the various farms, fitting the data from all farms to a statistical model was difficult. Therefore, a sub-sample of six farms was analyzed for differences in sample type on ergovaline and total loline concentrations. Data were analyzed as a completely randomized design with a one-way treatment structure.

Results

Concentrations, averaged across all sampling data, of ergovaline (Figure 1) and total lolines (Figure 2) in pure tall fescue clipped samples (TF) differed ($P < 0.01$; $n = 6$) from those taken inside (IN) and outside (OUT) a cherry tree drip line, as well as samples that were a forage/weed composite (COMP). Ergovaline concentrations in TF (0.29 $\mu g \cdot g^{-1}$) samples were more than two-fold higher than the average of those in the COMP, IN, and OUT (0.11 $\mu g \cdot g^{-1}$) treatment groups. Loline levels in TF (559 $\mu g \cdot g^{-1}$) were 7.5 times greater than the average of samples in the COMP, IN, and OUT (73 $\mu g \cdot g^{-1}$) treatments. Because clipped samples from IN and OUT treatments did not differ from COMP, only COMP and TF samples will be discussed for all other results.

At initiation (February) of the monitoring program, both ergovaline (Figure 3) and total loline (Figure 4) concentrations were below detectable levels. However, by March, average concentrations of ergovaline and total lolines steadily increased in both the TF and COMP treatments. The greatest increase in ergovaline levels was observed in TF samples, peaking at 0.6 $\mu g \cdot g^{-1}$during the month of May, whereas the maximum level of ergovaline in COMP samples was 0.2 $\mu g \cdot g^{-1}$. Average loline levels were consistently higher for TF samples as compared with COMP; however, changes in concentration throughout the monitoring program were relatively small.

Ergovaline and total loline levels in both TF and COMP samples, for all farms from April 15 to May 31, are given in Figures 5 and 6. A majority of early fetal losses (EFL) and late fetal losses (LFL) occurred during the month of May. Although results from this monitoring program cannot determine if endophyte-infected tall fescue is directly associated with MRLS, examining alkaloid concentrations

Figure 1. Ergovaline concentrations in pastures monitored for MRLS.

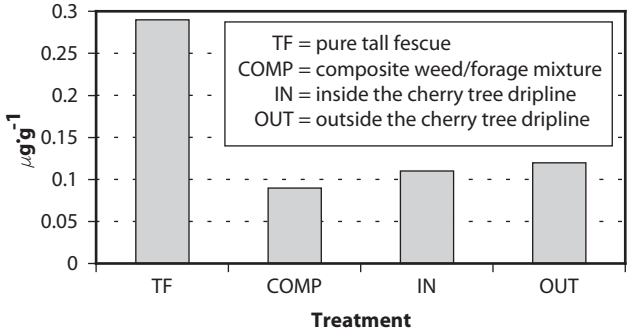


Figure 2. Loline concentrations in pastures monitored for MRLS.

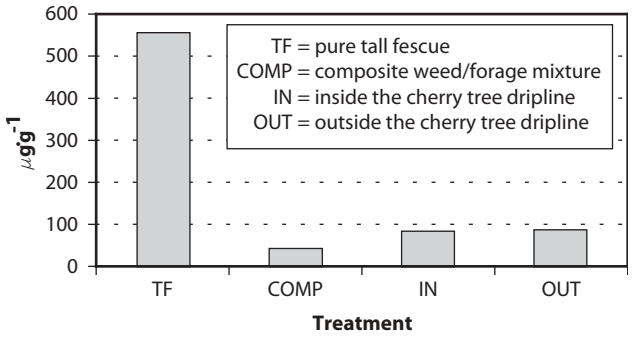


Figure 3. Bimonthly changes in ergovaline concentration.

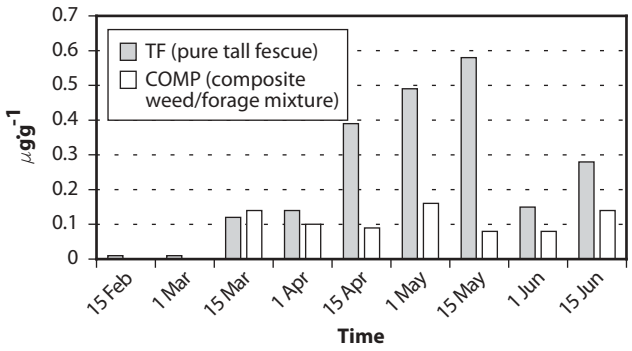
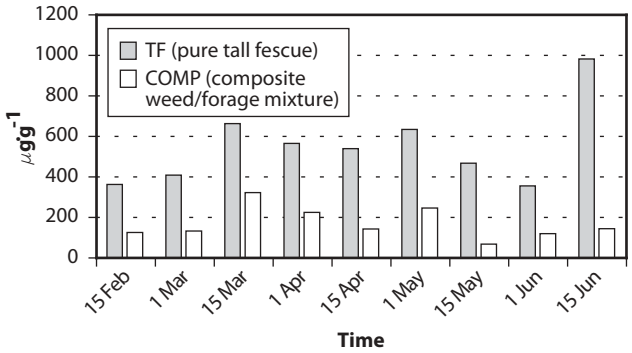


Figure 4. Bimonthly changes in total loline concentrations.



during the time of maximum foal losses is important. Alkaloid concentrations (ergovaline and total lolines) were consistently higher for TF samples as compared with COMP. Ergovaline in TF was between 0.25 and 0.7 $\mu g \cdot g^{-1}$ across all farms, whereas levels in COMP ranged between 0 and 0.23 $\mu g \cdot g^{-1}$. Average concentrations of ergovaline were 0.5 and 0.2 $\mu g \cdot g^{-1}$ for TF and COMP, respectively. Total loline concentrations were also consistently greater for TF versus COMP. Concentrations ranged from 250 to 720 $\mu g \cdot g^{-1}$ for TF and 7 to 225 $\mu g \cdot g^{-1}$ for COMP samples. Average loline levels were 543 versus 169 $\mu g \cdot g^{-1}$ for TF and COMP, respectively.

Discussion

Pure tall fescue clipped samples contained consistently higher levels of both ergovaline and total lolines than found in COMP samples. Minimal research is available on the effects of purified lolines or ergovaline on large animal performance. However, the negative endophyte-infected effects of tall fescue on performance and reproduction of horses are well documented (5,6,7,10). Ergovaline levels found in TF samples on all farms approached or were greater than the suggested 0.3 $\mu g \cdot g^{-1}$ concentration found in literature that results in decreased performance. In fact, greater than 50% (8 of 13) of all farms had ergovaline concentrations above 0.3 $\mu g \cdot g^{-1}$ during the month of May when fetal losses were greatest. However, it cannot be determined from these results if tall fescue was directly involved with any of the losses that occurred during that time. Also, concentrations of ergovaline in COMP samples were numerically lower than that found in TF. Composite samples were a forage/weed mixture with such grasses as tall fescue, timothy, orchardgrass, and bluegrass making up some of the forage species of the mixture. Horses are selective grazers, and the intake of tall fescue relative to total pasture ingested on a day-to-day basis was not recorded, making it difficult to estimate alkaloid intake.

Conclusion

With the type of monitoring program implemented, it is difficult to determine if endophyte-infected tall fescue played a role in MRLS. Further research is needed to properly answer that question. However, most importantly, one cannot discard the fact that high concentrations of ergovaline in horse pastures during late-term pregnancy can result in high foal mortality, decreased udder development and milk production, dystocia, and prolonged gestation. Monitoring mares during this time and removal from infected pastures at 300 days of gestation and subsequently after they have foaled and been rebred through 40 days of gestation have been suggested as a means of preventing the above reproductive complications (11).

Figure 5. Ergovaline concentrations for COMP and TF samples monitored for MRLS from April 15 to May 31.

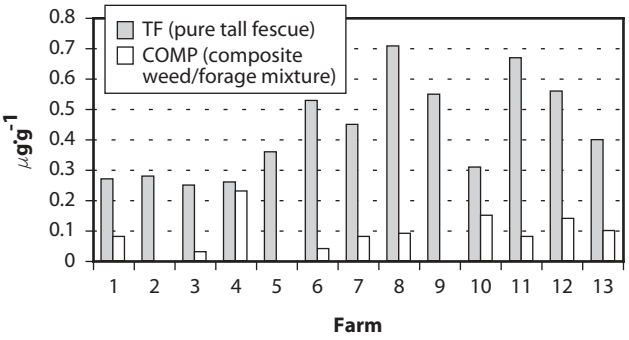
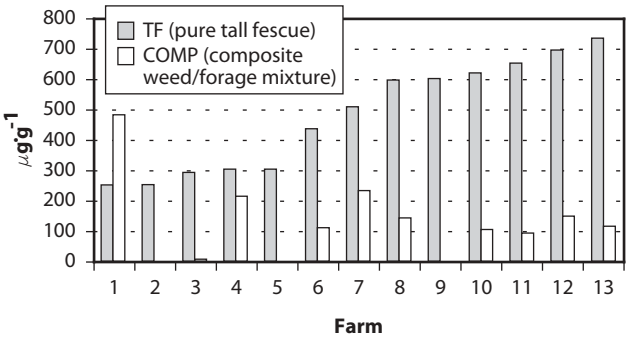


Figure 6. Total loline concentrations for COMP and TF samples monitored for MRLS from April 15 to May 31.



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Phytoestrogens and Estrogenic Activity in White Clover Samples from No-Loss and High-Loss Fields during Mare Reproductive Loss Syndrome, 2001

K. McDowell, R. Allman, and J. Henning

MARE REPRODUCTIVE LOSS SYNDROME (MRLS) WAS IDENTIfied in April of 2001 and thereafter on horse farms in Central Kentucky. It consisted of an excessive number of early fetal losses (EFL) and late fetal losses (LFL). During the spring of 2001, many samples were obtained from horse pastures and hay from Central Kentucky. Samples analyzed for phytoestrogens and estrogenic activity are the focus of this report.

Phytoestrogens are substances found in many plants that can interact with the estrogen receptor and elicit estrogenic effects (1-3). Common plants such as clovers, alfalfa, and soybeans contain phytoestrogens. Phytoestrogens in soybeans are reported to have sufficient estrogenic activity to relieve some of the symptoms associated with menopause (3). Estrogenic substances, such as estrogenic mycotoxins and phytoestrogens, have long been known to be deleterious to reproduction in swine and ruminants. They can increase interovulatory intervals in swine and decrease interovulatory intervals as well as cause embryonic and fetal abortions in ruminants (4-7).

In the 1940s, subterranean red clover caused abortions and other reproductive problems in sheep in Australia (8). The causative agents in the clover were the phytoestrogens biochanin A and formononetin.

While phytoestrogens are not known to cause reproductive problems in horses, the unique conditions in late spring of 2001 may have triggered an unusual combination of phytoestrogens to which the mares were exposed and possibly caused or contributed to MRLS. White clovers are the pasture plants most likely to contain phytoestrogens in horse pastures in Central Kentucky. Therefore, white clover in pasture and hay samples collected from fields where mares sustained high versus low incidences of fetal loss were analyzed for phytoestrogen content and estrogenic activity.

Materials and Methods

Between May 6 and June 6, 2001, 25 samples of white clover from pastures and hay were obtained from 10 different horse farms. The farms reported the degree of fetal loss for each field. Portions of all samples were sent to Dr. Patricia Murphy, Food Science and Human Nutrition Department, Iowa State University, Ames, Iowa, where they were analyzed for a panel of phytoestrogens. Additionally, portions of eight of the samples, representing three farms, were also sent to Dr. George Clark, Xenobiotic Detection Systems Inc., Durham, North Carolina, where they were analyzed for estrogenic activity. The analysis for estrogenic activity requires that samples be preserved by freezing immediately upon collection. Only the eight samples, representing three farms, were frozen upon collection and were thus deemed suitable for the analysis.

Results

More than 300 separate tests were performed for estrogenic activity and/or phytoestrogen content. Total estrogenic activity ranged from less than 1 ng/g sample to ap-

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proximately 10 ng/g (tested against the standard estradiol-17ß) (Table 1). There were no consistent differences among samples obtained from fields with mares reported to have high incidences of fetal loss versus those that reported little or no fetal losses.

The phytoestrogens included in the analyses were daidzin, genistin, glycitin, malonyl daidzin, malonyl genistin, malonyl glycitin, acetyl daidzin, acetyl genistin, acetyl glycitin, daidzein, genistein, glycitein, coumestrol, formononatin, and biochanin A (Table 2). The individual isomers of genistein, daidzein, and glycitein were not detectable in any of the samples. Formononetin was the only phytoestrogen that was consistently detectable, with levels on all but two samples ranging from 100 to 300 µg/g sample. The other two samples had the highest levels detected, at 1,037 and 3,295 µg/g sample. Those same two samples were the only samples with detectable levels of biochanin A, at 286 and 2,386 µg/g sample.

Table 1. Estrogenic activity of clover samples taken from high-loss and low-loss fields.

Farm ID	Reported Incidence of Fetal Loss for Field Sampled	Mean of Duplicate Estimates (ng/g)
A	severe	1.28
A	none	3.05
B	no report	5.32
A	severe	0.83
A	none	3.71
B	high	3.56
B	none	10.25
C	severe	10.72

Discussion

Formononetin and biochanin A were the only phytoestrogens that were consistently detectable in the samples of white clover obtained from selected horse farms in June of 2001. These are also the phytoestrogens found in subterranean red clover that caused the Australian sheep infertility problems of the 1940s. However, those concentrations were substantially higher, at approximately 10,400 and 19,000 µg/g sample, respectively. The concentrations found in our samples were consistently lower, regardless of degree of fetal loss associated with those fields. Additionally, they were below the concentrations in some reports for alfalfa sprouts sold for human foods (9,10).

Reported signs associated with MRLS include EFL and LFL as well as increased incidences of pericarditis and uveitis. Signs that one would associate with hyperestrogenism, such as increased interovulatory interval in nonpregnant mares, inappropriate estrous behavior, and inappropriate uterine edema were not reported as part of MRLS. Estrogens can be luteolytic in cattle and sheep and luteostatic in swine (4-7;11-13), but estrogens are not luteolytic or luteostatic in horses (14-16).

We cannot exclude the possibility that phytoestrogen levels might have been higher in horse pastures in the middle of April of 2001 when fetal losses were first detected than during May and June when our samples were collected. Neither can we exclude the possibility that phytoestrogens other than these 15 common ones were present in the samples that we tested. However, based on the low to nondetectable concentrations found in the samples reported here and that those samples represented fields from which mares sustained severely high to no fetal losses, it is unlikely that phytoestrogens as measured in these sample are responsible for MRLS.

Acknowledgments

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Table 2. Phytoestrogen content of clover samples taken from high-loss and low-loss fields.

Farm ID	Reported Incidence of Fetal Loss for Field Sampled	Total Dein[1]	Total Gein[2]	Total Gly[3]	Coumestrol –g/g sample	Formononetin –g/g sample	Biochanin –g/g sample
Assayed for estrogenic activity and phytoestrogen content							
A	severe	ND	ND	ND	175	145	ND
A	none	ND	ND	ND	ND	376	ND
B	no report	ND	ND	ND	ND	174	ND
A	severe	ND	ND	ND	ND	156	ND
A	none	ND	ND	ND	ND	102	ND
B	high	ND	ND	ND	ND	96	ND
B	none	ND	1	ND	ND	174	ND
C	severe	ND	ND	ND	ND	172	ND
Assayed for phytoestrogen content only							
D	severe	ND	ND	ND	ND	250	ND
E	moderate	ND	ND	ND	ND	3295	2386
E	moderate	ND	ND	ND	ND	198	ND
F	severe	ND	ND	ND	ND	246	ND
G	severe	ND	ND	ND	ND	282	ND
H	none	ND	ND	ND	ND	223	ND
I	light	ND	ND	ND	521	388	ND
A	severe	ND	ND	ND	ND	240	ND
A	severe	ND	ND	ND	ND	276	ND
A	none	ND	ND	ND	367	225	ND
A	none	ND	ND	ND	ND	153	46
J	moderate	ND	ND	ND	ND	308	ND
hay	not applicable	ND	ND	ND	ND	222	ND
hay	not applicable	ND	ND	ND	ND	204	ND
hay	not applicable	ND	ND	ND	ND	1037	286
hay	not applicable	ND	ND	ND	ND	161	ND
hay	not applicable	ND	ND	ND	ND	260	ND

[1] Total Dein is the sum of the individual isomers of daidzein and includes daidzin, malonyl daidzin, acetyl daidzin, and daidzein.

[2] Total Gein is the sum of the individual isomers of genistein and includes genistin, malonyl genistin, acetyl genistin, and genistein.

[3] Total Gly is the sum of the individual isomers of glycitein and includes glycitin, malonyl glycitin, acetyl glycitin, and glycitein.

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Review of Mycotoxins as a Possible Cause of Mare Reproductive Loss Syndrome

K. Newman

THE ROLE OF MYCOTOXINS IN MARE REPRODUCTIVE LOSS Syndrome (MRLS) has been a primary focus of the investigations since early in May of 2001. At that time, a number of forage samples were tested for the mycotoxin zearalenone and found to contain quantities ranging from 200 to 1,500 parts per billion (ppb). Since zearalenone has been demonstrated to cause abortions in a number of animal species, the focus on mycotoxins as being one of the primary suspects in this investigation seemed quite justified. Combine this with the fact that drought and temperature stress can evoke toxin production from certain molds (1,2) and the fact that the spring of 2001 was a very dry, warm period followed by a dramatic drop in temperature leading to frost occurring on April 17 and 18, and it seemed quite cut-and-dried that the cause of MRLS was mycotoxin intoxication. Mycotoxins are not transmissible from animal to animal and do not respond to drug and antimicrobial therapy. In addition, most mycotoxins are toxic but not lethal at environmental levels (example: zearalenone has an LD_{50} greater than 16 g/kg of body weight). Zearalenone has estrogenic properties, although it is not chemically an estrogen. For this reason, females are traditionally more susceptible than males. In swine, a swollen, red vulva, which may lead to rectal and vaginal prolapse, is normally only seen in prepubertal gilts (3). In MRLS, maiden and previously barren mares seemed to be more susceptible to the syndrome. A number of studies in other animal species have demonstrated the effects of zearalenone over extended periods of time; a more probable scenario under field conditions (and in examining MRLS) is dosing of toxin over a short period of time. Studies with 16 gilts given pure zearalenone (108 mg on post-mating days 2 to 6, 7 to 10, or 11 to 15) showed that gilts given zearalenone on post-mating day 7 to 10 had reduced embryonic survival than control or other treatment groups (1 of 4 pregnant in the 7 to 10 day group; 4 of 4 pregnant in control and other treatment groups). FSH and estradiol-17ß concentrations were unaffected by zearalenone consumption. Serum concentrations of prolactin in the 7 to 10 and the 11 to 15 group were lower than control or the 2 to 6 post-mating groups (4).

Similar problems associated with abortions in cattle have also been attributed to zearalenone-contaminated hay (5). Because of the critical effects of zearalenone on stage of pregnancy, it was thought that this toxin may have played a critical role in the events described in the spring of 2001. This may account for the observation of affected mares being 40 to 100 days pregnant or near term.

In addition, North Carolina State University (NCSU) and the University of Guelph last year (personal communication) have presented data demonstrating the presence of a significant amount of different mycotoxins in forage samples. In the study from NCSU, they detected aflatoxin, deoxynivalenol (DON), trichothecene (T-2), and zearalenone (ZEA) in forage (Table 1). It is important to emphasize that, in both the Guelph and NCSU trials, commercial ELISA test kits were used for the analysis.

Table 1. Mycotoxins present in forage samples as detected by commercial ELISA test kits.

	Aflatoxin	DON	Fumonisin	T-2	Zearalenone
Detected	Yes	Yes	No	Yes	Yes

(6)

In the case of MRLS investigations, the early results demonstrating the presence of zearalenone were also determined by ELISA kits. These kits are not validated for the complex matrices of forage, caterpillar, and caterpillar frass samples. ELISA procedures are prone to false positive results on nonvalidated matrices and should be confirmed using high performance liquid chromatography (HPLC) and/or thin layer chromatography (TLC) testing. When confirmation of these positive results was requested, one of the major problems was the availability and quantity of samples from the critical time frame. However, no Fusarium toxins were detected in any of the samples submitted for HPLC analysis. What was once a simple task of validation of what was already suspected turned to a "what do we do now?" situation.

Since the presence of mycotoxins is often very difficult to detect under the best circumstances, the inability to confirm their presence in the few samples available for duplicate analysis by other methods and the failure to detect them in forage samples taken after the critical insult period does not eliminate mycotoxins as a possible cause for MRLS. As with any investigation, the need to follow up on the clues presented at the time was the next step. An important observation came from Dr. Bruce Webb,

Venture Laboratories Inc., Lexington, Kentucky.

an entomologist at the University of Kentucky. From his extensive work on caterpillars, he observed that caterpillar frass was an excellent growth medium for molds. From this observation, a theory was developed that caterpillar frass served as a "fertilizer" for mold growth and mycotoxin production leading to MRLS. Field samples taken during the early summer of 2001 confirmed the observation of frass supporting mold growth, with *Aspergillus*, *Fusarium*, and *Penicillium* sp. being the predominant genera isolated (Table 2).

Table 2. The ability of ETC frass to support the growth of various fungi taken from environmental samples.

Environmental Fungal Isolate	Growth on Frass
Aspergillus flavus	Yes
Aspergillus fumigatus	Yes
Fusarium sp.	Yes
Fusarium graminearum	Yes
Fusarium poae	Yes
Penicillium sp.	Yes

Taking this theory one step further, it was observed that *Penicillium* sp. dominated the mycoflora of cultures of ETC and frass from trials where caterpillars were either mixed in the feed or intubated in pregnant mares and subsequently caused symptoms consistent with MRLS (Table 3). Caterpillar frass samples contained approximately 100 times higher concentrations of penicillium than the caterpillar samples (10^7 CFU/g versus 10^5 CFU/g). These concentrations of fungi supported the theory that frass was an excellent growth medium for fungi; however, with the exception of the first mare trial, frass alone has not been shown to cause mares to abort. *Penicillium* sp. have been isolated from every frass sample (seven) obtained in 2002. Fresh caterpillars (taken from trees less than 30 minutes prior to testing) demonstrate $<10^3$ CFU/g of mold from the gastrointestinal tract contents. However, *Penicillium* sp. have been consistently isolated from microbial examinations of entire caterpillars from spring of 2002, with *Aspergillus* and *Fusarium* sp. making up the vast majority of the mold populations from these samples. No known mycotoxins were identified from any of the caterpillar homogenates.

Substantiating the frass fertilizer theory was a recent publication that showed uric acid enhancing toxin production from penicillium molds (7). Uric acid is a primary component of frass (4 to 6%) and seems to be a good nitrogen source for mold growth and a catalyst for production of certain toxins. Published *in vitro* trials looked at different uric acid concentrations and the ability of uric acid to improve alkaloid production by penicillium mold.

In our own observations, uric acid also stimulated the growth rate of five separate *Penicillium* sp. (5 to 30% increase) and three *Fusarium* sp. (7 to 21% increase). Since a period of very warm weather followed by frost was observed in the spring of 2001, and frost exposure being a catalyst for toxin production of certain molds, an examination of the effects of frost on environmental isolates from predominant mold isolates seemed a logical additional piece of required evidence. As expected, exposure to freeze-thaw situations increases toxin production especially in the case of *F. poae* (30% increase).

Table 3. The concentrations of fungi observed from samples of ETC and ETC frass from environmental sources.

Sample	Mold Concentration	Predominant Mold Genera
Frass from intubation trial	1.2×10^7 CFU/g	Penicillium
Frass from affected farm (A)	1.2×10^9 CFU/g	Penicillium; Aspergillus
Frass from affected farm (B)	9.5×10^7 CFU/g	Penicillium
Frass from environmental samples (n = 4)	$4.0 - 6.6 \times 10^8$ CFU/g	Penicillium, Fusarium
Caterpillar GI tract contents	$<10^3$ CFU/g	Not Applicable
Caterpillars from intubation trial	1.7×10^5 CFU/g	Penicillium; Mucor
Intact, entire caterpillars	1.5×10^5 CFU/g	Penicillium

From a scientific standpoint, the circumstantial evidence was beginning to build for a possible mycotoxin insult being responsible for MRLS. Uric acid was shown to stimulate mold growth and toxin production with frost conditions apparently exacerbating toxin production. Then, two separate trials by B. Webb et al. and B. Bernard et al. (this proceedings) found that caterpillars, not frass, were associated with symptoms consistent with MRLS under controlled conditions. Samples of these caterpillars were tested for mycotoxins using a combination of TLC, ELISA, and HPLC and found to be below detectable limits for the major known fusarium, penicillium, and aspergillus mycotoxins. If the data presented from the trials of the above are valid, then mycotoxins are not responsible for MRLS fetal loss. This is a logical finding considering that it seems highly unlikely that a caterpillar would somehow sequester mycotoxins either in it or on it (no known fusarium, penicillium, or aspergillus toxins were detected from caterpillar homogenates). This does not eliminate other biological toxins from being involved in MRLS fetal loss, pericarditis, or eye problems. A variety of microorganisms have been associated with tent caterpillars. The identity of these organisms and possible toxic agents associated with them warrants further investigation. It is also possible that there may be a role for mycotoxins in pericarditis and/or eye problems that were also observed in a small percentage of the horse population during the same time period. There are data in the literature to support a role for mycotoxins in cases of unilateral blindness and heart problems in a variety of species.

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Cherry Trees, Plant Cyanogens, Caterpillars, and Mare Reproductive Loss Syndrome: Toxicological Evaluation of a Working Hypothesis

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THE ONSET OF MARE REPRODUCTIVE LOSS SYNDROME (MRLS) in 2001 coincided with an exceptional abundance of eastern tent caterpillars (ETC), *Malacosoma americanum,* in Central Kentucky. Preliminary field studies by Henning and his co-workers also showed a high geographic correlation between ETC, black cherry trees, and MRLS. This correlation was strongly confirmed by a later epidemiological survey by R. Dwyer et al. (1). Further support for this association was provided by a toxicology report of high cyanide concentrations in the hearts of three late fetal loss (LFL) foals (L. Harrison, personal communication).

The MRLS 2001 syndrome was a completely new entity, having never been identified or described previously. It was also extremely transient, appearing and peaking in less than two weeks. As such, for the remainder of 2001, the syndrome was viewed, analyzed, and researched in an increasingly retrospective manner.

It was soon hypothesized that cyanide from black cherry tree leaves was the proximal cause of MRLS. Cyanide or cyanogens were thought to be transferred from the trees to the environment of the horse by ETC voraciously denuding these trees (Figure 1). The precise mechanism of the transfer was unclear. The source(s) of cyanogens could be the caterpillars themselves, caterpillar frass, leaf fragments, mandelonitrile ingestion, water contaminated by caterpillars, combinations of these factors, or by other sources such as pasture clover. The concentrations of cyanide involved were assumed to be sub-lethal for mares since mares did not show clinical signs, and it was assumed that the fetus was more sensitive than the mare to cyanide. If the hypothesis was correct, it should be possible to abort mares by exposing them to sub-clinical concentrations of cyanide.

Since there was virtually a complete absence of published literature about cyanide in the horse, let alone the pregnant mare, the objectives of this study were to define "normal" cyanide concentrations in equine blood in Central Kentucky, determine the "threshold" toxic level for cyanide toxicity in equine blood, and determine fetotoxicity of cyanide in the pregnant mare. The overall goal of this project was to reproduce MRLS in the laboratory.

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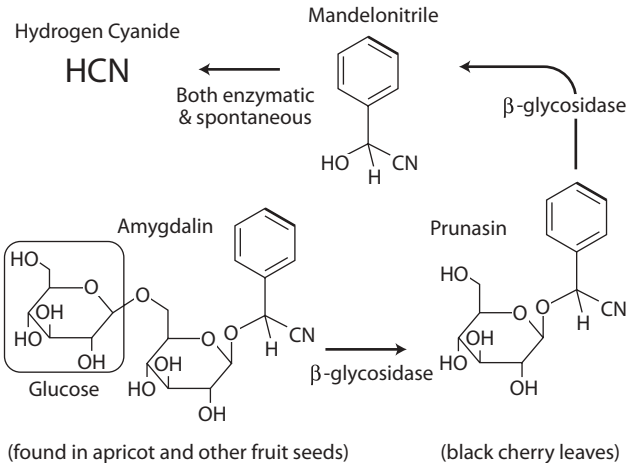
Figure 1. Cyanogenic cascade in black cherry (prunasin) and apricot (amygdalin). Both cascades proceed through prunasin, and mandelonitrile is the proximal cyanide donor.



Materials and Methods

Horses

Mature Thoroughbred mares weighing 428 to 504 kg were used for this study. The animals were maintained on grass hay and feed (12% protein), which was a 50:50 mixture of oats and an alfalfa-based protein pellet. Horses were fed twice a day. The animals were vaccinated annually for tetanus and were de-wormed quarterly with ivermectin (MSD Agvet, Rahway, NJ). A routine clinical examination was performed before each experiment to assure that the animals were healthy and sound. Additionally, cardiac and ophthalmic evaluations were performed to ensure that those organs had no evidence of previous disease. All animals used in these experiments were managed according to the rules and regulations of the University of Kentucky Institutional Animal Care Use Committee, which also approved the experimental protocol.

Cyanide Infusion

An intravenous catheter (Abbocath-T, 14g x 5½", North Chicago, IL) was inserted into the jugular vein and sutured in place. Sodium cyanide (NaCN) solutions were prepared by dissolving NaCN (J.T. Baker, Philipsburg, NJ) in saline and were infused at 3, 6, and 12 mg/minute for 1 hour and 1 mg/kg for 1 hour using an ambulatory withdrawal pump (Dakmed, Buffalo, NY). During infusion, the horses were monitored closely for signs of toxicity, which included restlessness, anxiety, flared nostrils, rapid respiration, sweating, and increased heart rate. Heart rates (HR) were recorded at 1-minute intervals by an onboard heart rate computer (Polar CIC Inc., Port Washington, NY). For the 3, 6, and 12 mg/minute infusions, blood samples were taken before and at various times during and after infusion for complete blood counts, chemistry panels, and blood lactates. For the 1 mg/kg for 1 hour infusion, blood samples were obtained for analyses before infusion (0 hours), during infusion (0.25, 0.5, and 1 hour) and after infusion (0.08, 0.17, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144 hours) into Vacutainer® serum tubes (Becton Dickinson, Rutherford, NJ) and Vacutainer® plasma tubes (Becton Dickinson, Franklin Lakes, NJ) and analyzed immediately.

Mandelonitrile Administration

In a second series of experiments, four mature Thoroughbred mares were administered oral mandelonitrile (3 mg/kg). Blood samples for cyanide analysis were obtained before (0 hours) and after dosing at 0.05, 0.08, 0.17, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours into Vacutainer serum tubes and Vacutainer plasma tubes and analyzed immediately.

Safety Precautions

Two antidotes were prepared to counter any adverse effects from cyanide infusion. A 3% solution of sodium nitrite was prepared by adding 1.8 g of Na nitrite (J.T. Baker, Philipsburg, NJ) to 60 ml of saline. This mixture was to be administered intravenously at a rate of 10 to 20 ml/minute. A 25% solution of sodium thiosulfate (J.T. Baker, Philipsburg, NJ) was prepared by adding 100 g Na thiosulfate to 400 ml of saline. This mixture was to be administered immediately after the sodium nitrate at a rate of 200 ml/minute.

Analytical Detection of Cyanide

As detailed previously (1), an inexpensive, disposable alternative to the costly Warburg Distillation Flask was developed, which allowed simultaneous running of 100 cyanide analyses. Briefly, a 10-ml plastic cup was suspended by means of Scotch® tape inside a 120-ml plastic cup with a screw lid; 10 ml of 1 molar sulphuric acid (H_2SO_4) was pipetted into the larger cup. Exactly 2.5 ml of 0.25 normal sodium hydroxide (NaOH) was pipetted into the smaller cup. The cyanide-containing sample (e.g., 1 to 2 ml blood) was pipetted into the H_2SO_4, and the cup was immediately sealed with its lid and allowed to sit overnight at room temperature while cyanide as HCN gas was evolved from the acid solution and trapped in the NaOH. The small cup was then removed, and the NaOH solution was decanted into an autoanalyzer sample cup. In the presence of chloramine-T, the cyanide ion was converted to cyanogen chloride, which reacted with pyridine-barbituric acid to form a red-blue color, the intensity of which was measured spectrophotometrically at 578 nm. Use of an autoanalyzer ensured a precise and reproducible interval during which color developed and thus improved the detection limit to as low as 2 ng/ml in a 1-ml sample.

Standard curves were linear in the range of 2 to 300 ng/ml, with a regression coefficient $r^2 > 0.99$.

Toxicokinetic Analysis

The toxicokinetic parameters of cyanide were determined by compartmental analysis. Equations of a two-compartment model with zero-order input rate were fitted to the individual blood concentrations versus time by least squares nonlinear regression analysis using a nonlinear regression program (Winnonlin, version 3.1) (Pharsight Corporation, Cary, NC). The closeness of the fit was evaluated by the Akaike Information Criterion (AIC), residual plots, and visual inspection. The data were weighted as $1/(y_{pred})^2$, where y_{pred} was the model-predicted concentration at the actual time. Area under the curve (AUC) following intravenous administration was measured by use of a linear trapezoidal approximation with extrapolation to infinity, and slope of the terminal portion (ß) of the log plasma drug concentrations versus time curve was determined by the method of least-squares regression (2). The rate constant of distribution α and distribution half-life ($t_{1/2}\alpha$) were determined using the method of residuals (2). Total body clearance (Cl_s) was calculated by use of Equation 1 (3).

$$Cl_S = \text{IV Dose}/AUC_{0\text{-inf}} \qquad \text{(Equation 1)}$$

The volume of distribution in central compartment (Vd_c) and volume of distribution at steady state (Vd_{ss}) were calculated according to Equations 2 and 3, respectively (4).

$$Vd_c = \text{Dose (IV)}/(A+B) \qquad \text{(Equation 2)}$$

$$Vd_{SS} = \text{IV Dose}/AUC_{0\text{-inf}} \times MRT \qquad \text{(Equation 3)}$$

A and B are the Y intercepts associated with distribution and elimination phase, respectively, and AUMC is area under the first moment curve and calculated by the trapezoidal method and extrapolated to infinity.

The mean residence time (MRT) (5) was determined according to Equation 4.

$$MRT = AUMC_{0\text{-inf}}/AUC_{0\text{-inf}} - (\text{Infusion time}/2) \qquad \text{(Equation 4)}$$

The pharmacokinetic variables (elimination half-life, area under the curve) of the cyanide following oral administration of mandelonitrile were determined using a noncompartmental approach (2). The maximum blood concentration of the cyanide (C_{max}) and the time to reach this concentration (T_{max}) were obtained directly from the blood-concentration versus time curves. The absolute bioavailability (F) was calculated from the $AUC_{0\text{-inf}}$ ratio obtained following oral and IV administration according to Equation 5 (4).

$$F = AUC_{0\text{-inf}}(\text{Oral})/AUC_{0\text{-inf}}(\text{IV}) \times \text{IV Dose/Oral Dose} \qquad \text{(Equation 5)}$$

Total oral clearance (Cl_o) was calculated by use of Equation 6.

$$Cl_O = \text{Dose (Oral)}/AUC_{0\text{-inf}} \qquad \text{(Equation 6)}$$

Results and Discussion

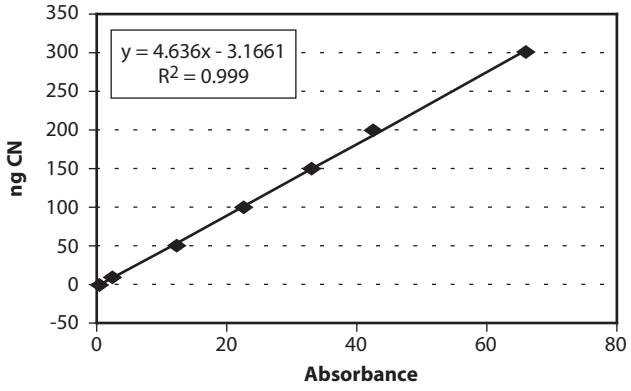
Developing a Highly Sensitive Analytical Method

Most analytical methods for cyanide are used in the forensic detection of cyanide associated with lethal intoxication and have a limit of detection in the mg/ml range. However, it was obvious that if cyanide was involved in MRLS, the concentrations found in mares would be less than those associated with clinical signs of toxicity since no signs of intoxication were noted in any mares. Therefore, an analytical method was needed for cyanide detection in the subtoxic range (ng/ml). Such a method was developed in our laboratory and has been described previously (6). Figure 2 shows the standard curve of that method for the quantification of cyanide in biological fluids.

Defining "Normal" Blood Cyanide in Horses at Pasture in Kentucky

The first step in this project was to define what constitutes a "normal" blood cyanide for a mare at pasture in Central Kentucky. Figure 3 shows that the distribution of blood cyanide concentrations in horses at pasture in Kentucky averaged 8.5 ng/ml in the fall of 2001 (n = 48) and 3.2 ng/ml in the spring of 2002 (n = 100). The blood cyanide concentrations apparently follow log normal distributions, and the concentrations appeared to differ from field to field and also between fall and spring. These blood

Figure 2. Standard curve for the quantification of cyanide in biological fluids.



cyanide concentrations are very low, and it should be kept in mind that cyanide is substantially concentrated in blood. Free blood cyanide and presumably free tissue concentrations of cyanide in horses at pasture in Kentucky are likely about 1/50 of these concentrations (7).

Defining "Toxic" Blood Concentrations of Cyanide in the Horse

The next step was to define the blood concentrations of cyanide likely to be acutely toxic in the horse. To this end, horses were infused with increasing concentrations of cyanide, starting at 1 mg/minute and increasing to 12 mg/minute. Figure 4a shows the blood cyanide concentrations attained following infusion of 12 mg NaCN/minute, the dose that produced the first signs of toxicity. As shown in Figure 4b, there was a sharp increase in heart rate at the point of toxicity during the 12 mg/minute infusion, when the heart rate peaked at 150 beats per minute (bpm). Other signs of toxicity were sweating, rapid breathing, and apparent anxiety.

When clinical signs of toxicity appeared, the infusion was immediately stopped. Thereafter, the heart rate returned to normal, and the horse quickly returned to normal behavior without administration of an antidote. In earlier experiments, cyanide toxicity would sometimes cause the horse to weaken and drop to the ground. These clinical signs were always rapidly reversed following intravenous infusion of 3% sodium nitrite and 25% sodium thiosulfate.

These experiments suggested that if the experimental blood cyanide concentrations were held to less than 2,000 ng/ml, clinical signs of cyanide toxicity in the experimental horses were unlikely. Additionally, these results suggest that there is a large difference between the concentration of blood cyanide in normal horses at pasture and the concentration required for toxicity allowing for considerable experimental latitude to evaluate the fetotoxicity of cyanide in pregnant mares.

Defining the Toxicokinetics of Cyanide in the Horse

Based on the data of Figure 4, we infused four horses with NaCN at 1 mg/kg for 1 hour. Blood cyanide concentrations increased linearly to about 1,000 ng/ml at 1 hour, at which point the infusion was stopped. After infusion was stopped, there was rapid redistribution of cyanide with an alpha half-life of 0.74 hours, followed by a beta or terminal phase of metabolism, which was much slower, with a half-life of 16 hours (Figure 5). The insert shows the logarithmic plot of concentration versus time for the post-administration portion of the data. These data show clearly that the termination of action after a bolus administration of cyanide is by re-distribution and that cyanide is a classic agent for which its acute pharmacological/toxicological effects can be terminated by redistribution.

Figure 3. Distribution of blood cyanide concentrations in horses at pasture in Kentucky a) in the fall of 2001 (n = 48) and b) in the spring of 2002 (n = 100).

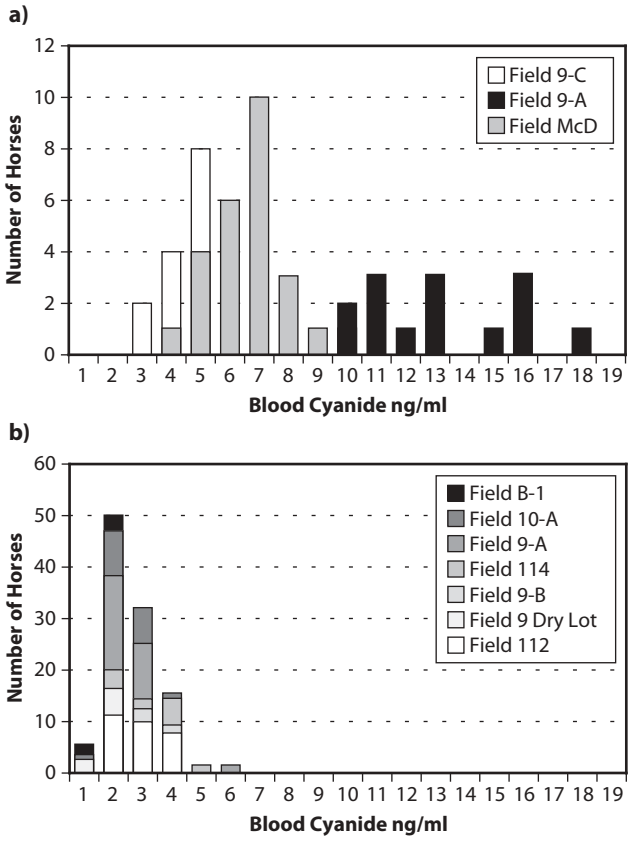


Figure 4. a) Mean blood cyanide concentrations attained following intravenous infusion of 12 mg NaCN/min, the dose that produced the first signs of toxicity; b) Heart rate of infused horse showing the point of toxicity, when the heart rate peaked at 150 bpm.

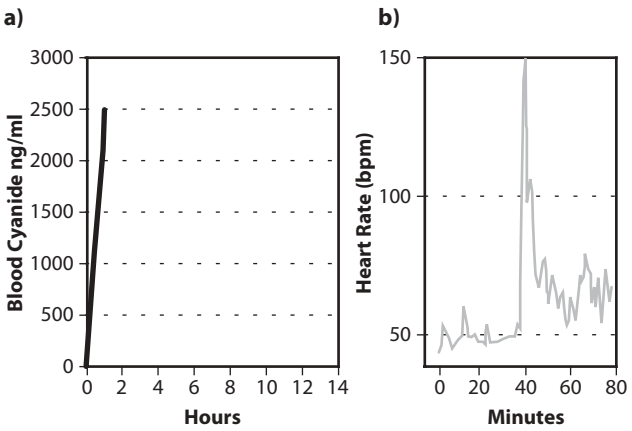
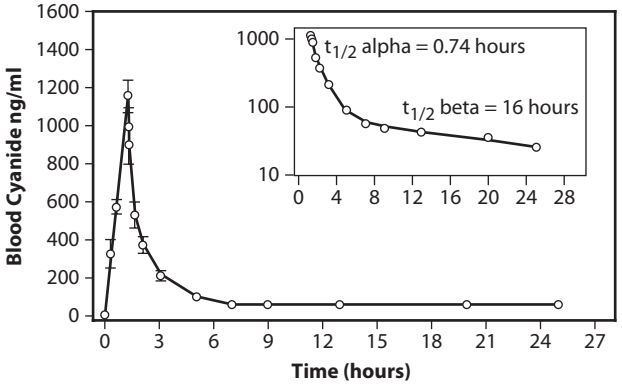


Figure 5. Blood cyanide concentrations following NaCN infusion at 1 mg/kg x 1 hour. Insert shows the logarithmic plot of concentration versus time for the post-administration portion of the data with an alpha half-life of 0.74 hours, followed by a terminal phase, with a half-life of 16 hours.

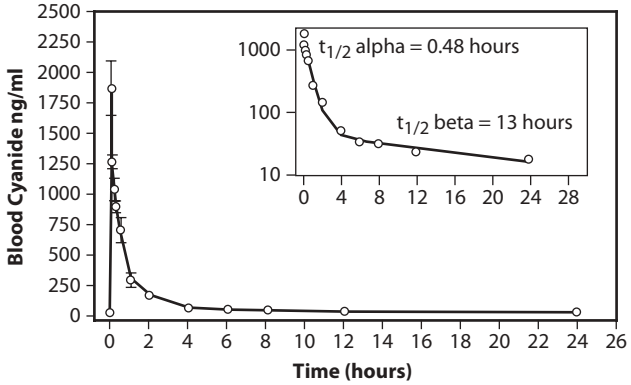


Defining the Toxicokinetics of Mandelonitrile in the Horse

Cyanide is not present as such in black cherry tree leaves but rather as prunasin and mandelonitrile, as set forth in Figure 1. Mandelonitrile is the proximate precursor in the cherry tree system for cyanide release. Therefore, to more closely mimic the possible etiology of MRLS, horses were dosed with oral mandelonitrile, and the resulting blood cyanide concentrations were measured. Figure 6 shows the mean blood cyanide concentrations following dosing with 3 mg/kg mandelonitrile (n = 4). Mandelonitrile is about 20% cyanide; therefore, horses received about 600 mg HCN orally. There was immediate release and absorption of cyanide as indicated by the peak blood cyanide concentration at 3 minutes. In an earlier ranging experiment, in which a dose of 3 g/horse of mandelonitrile was used, the test horse became weak and uncoordinated for about 30 seconds.

The insert of Figure 6 shows a semi-logarithmic plot of cyanide concentration versus time for the post-absorption portion of the data. The apparent bioavailability of cyanide from mandelonitrile was about 57%; absorption was followed by rapid redistribution of cyanide, with an alpha half-life of 0.48 hours. This phase was followed by the beta or elimination phase with a much slower half-life of about 13 hours.

Figure 6. Mean blood cyanide concentrations following bolus oral dose of 3 mg/kg mandelonitrile (n = 4). Insert shows semi-logarithmic plot of cyanide concentration versus time for the post-absorption portion of the data. Absorption was followed by rapid redistribution of cyanide, with an alpha half-life of 0.48 hours and a beta phase half-life of about 13 hours.



Attempted Reproduction of MRLS by Administration of Mandelonitrile to Pregnant Mares

We next attempted to reproduce MRLS in pregnant mares by administration of mandelonitrile. With regard to developing a suitable dosing schedule for mandelonitrile, our pharmacokinetic results showed that it would be difficult to maintain a "steady-state" plasma concentration of cyanide following intermittent oral dosing with mandelonitrile. As a best experimental approach, seven pregnant mares were dosed with mandelonitrile (2 mg/kg twice a day in applesauce) for 14 days. Peak values (taken immediately after dosing) had a mean of 315 ng/ml and varied widely (range: 215 to 594 ng/ml) as indicated by the upper curve. The trough values (taken just before dosing) had a mean of 62 ng/ml and were more consistent than peak values (Figure 7).

Throughout this experiment, no fetal losses were recorded, and no clinical signs suggestive of fetal loss were observed. These results suggested that consistent blood concentrations of cyanide of about 60 ng/ml and/or considerably higher spikes of blood cyanide can occur without associated fetal losses. As such, these results are inconsistent with and do not support the original working hypothesis that cyanide from the black cherry tree is closely associated with or a proximal cause of MRLS.

Other Factors Possibly Influencing Blood Cyanide Concentrations

As the 2002 MRLS season approached, we paid particular attention to the possible impact of pasture clover content and overnight freezing conditions on the cyanide content/bioavailability of clover cyanide and the blood concentrations of cyanide in horses grazing such pastures. Because damage from freezing temperatures has been reported to increase the cyanide content and/or its bioavailability from some clovers, James Crutchfield and his colleagues studied the cyanide content/yield of clover and the effects of morning temperature. As shown in Figure 8, morning temperature had little effect on Kentucky clover cyanide content/yield, in contrast with the reported effects of low temperatures on cyanide content/yield from

Figure 7. Peak and trough blood cyanide concentrations following oral dosing of mandelonitrile (2 mg/kg, twice a day, in applesauce) for 14 days.

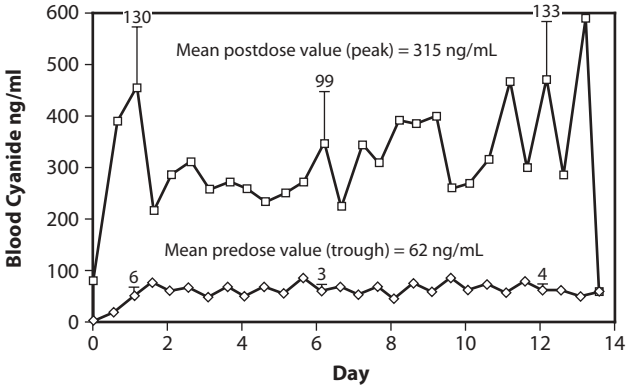
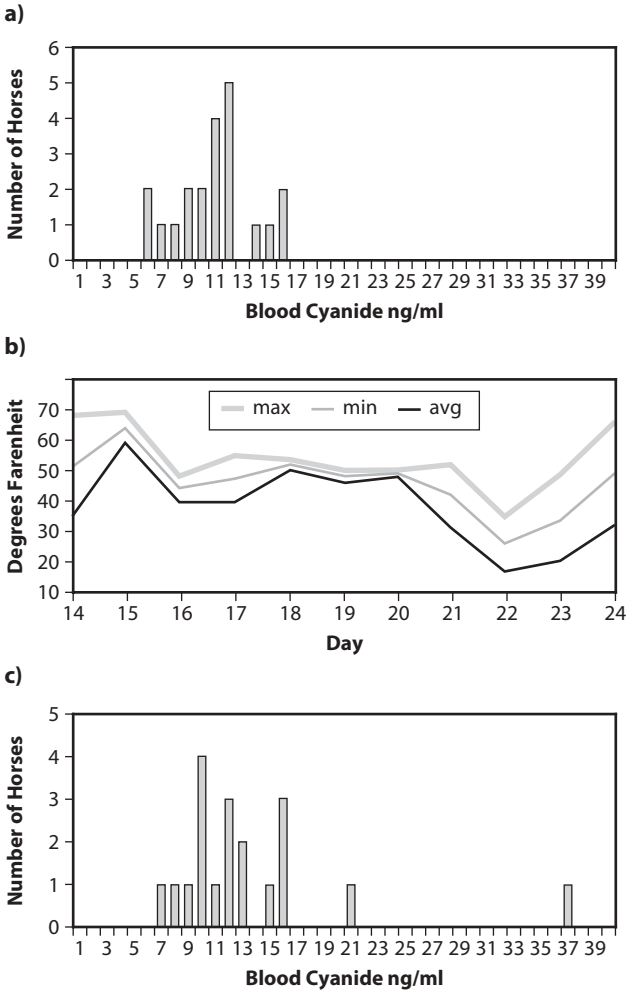


Figure 8. a) Blood cyanide concentrations in horses at pasture before a "freeze" (March 21); b) minimum, maximum, and average temperatures during mid-March; c) blood cyanide concentrations of same horses on March 22, after hard freeze.



clovers in other geographic locations. There was no significant difference between the blood cyanide concentrations before and after freezing.

Consistent with these findings, blood cyanide concentrations in horses at pasture showed little change following an overnight frost. Figure 8a shows the blood cyanide concentrations in horses at pasture before a "freeze" on March 21, and Figure 8c shows the blood cyanide concentrations of the same horses on March 22 after the hard freeze of the night of March 21. Figure 8b shows the minimum, maximum, and average temperatures during mid-March. Consistent with the results of the Crutchfield group experiments, there was no apparent difference in blood cyanides between each group of horses prior to or after the freezing night of March 21, 2002.

Field and Experimental Results from the 2002 MRLS Season

The interpretation of these experimental results is consistent with field and experimental data developed during the 2002 MRLS season. During the 2002 MRLS season, blood samples were drawn from a band of 20 pregnant mares grazing in proximity to black cherry trees. As set forth in Figure 9, the blood cyanide concentrations of these mares was not significantly different from "normal" mares shown in Figure 3. Furthermore, two of these mares (indicated with asterisks on Figure 9) had early fetal losses (EFL) consistent with MRLS, even though blood cyanide concentrations in these two mares were well within normal limits.

In cooperation with Drs. Webb and McDowell, we also monitored blood cyanide concentrations in mares undergoing ETC-induced EFL. Blood cyanide concentrations of mares after ETC exposure on May 29 were not significantly greater ($p < 0.05$) than blood cyanide concentrations before exposure on May 17 to ETC (Figure 10). Again, the results do not support suggestions that increased blood cyanide concentrations are a factor in ETC-induced fetal losses.

Figure 9. Blood cyanide concentrations of mares grazing in proximity to wild cherry trees from April 28 - May 3, 2002. Asterisks denote blood cyanide concentrations of two mares that suffered EFL during this period.

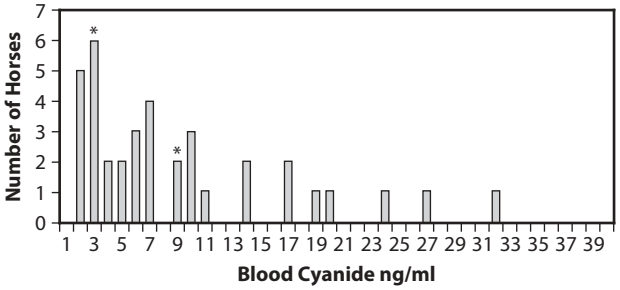
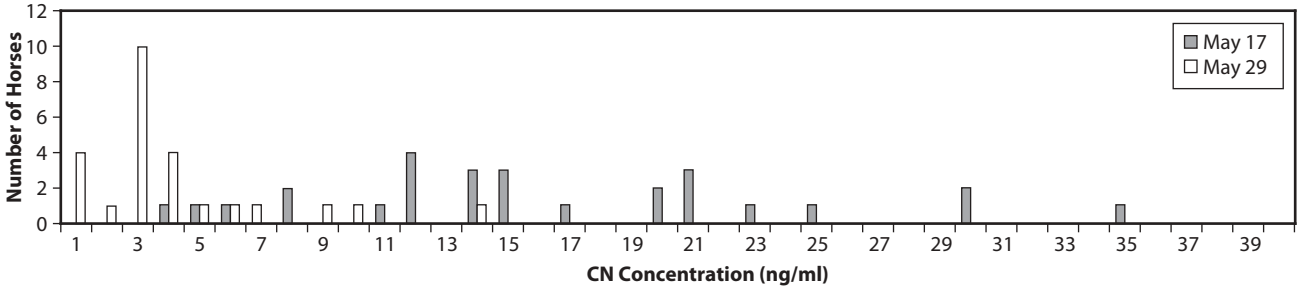


Figure 10. Blood cyanide concentrations of pregnant mares before exposure (May 17) and after exposure (May 29) to ETC.



Conclusions

Field and experimental events reported elsewhere in this workshop from the 2002 MRLS season have provided strong evidence in support of the close involvement of ETC in MRLS. On the other hand, the experimental and field work with cyanogens leading up to the 2002 MRLS season and results obtained during the 2002 MRLS season reported here make the involvement of cyanide in MRLS very much less likely.

Moreover, a study tracing the movement of cyanide from cherry trees to tent caterpillars and into the detritus pool sheds additional doubt on the possible involvement of caterpillar-borne cyanide in MRLS (8).

Simply put, we have been unable to reproduce MRLS by administration of mandelonitrile, a proximal cyanide donor, and no evidence whatsoever has been developed in support of suggestions that exposure to black cherry trees, ETC, or cyanogenic clovers was likely to increase the blood cyanide concentrations of mares to the point that such blood cyanide concentrations could be implicated in MRLS.

In further support of this conclusion, MRLS was seen to occur in mares in which blood cyanide concentrations were no different from or actually less than those seen in "normal" or "control" mares, consistent with suggestions that cyanide from ETC or any other source has not been a critical factor in MRLS as we know it.

Acknowledgments

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Summary

T. Tobin

MARE REPRODUCTIVE LOSS SYNDROME (MRLS) SEEMS TO BE strongly associated with the caterpillars. If you put the caterpillars near horses, or in horses, or, as was described here this morning, if the horses eat the caterpillars—and it seems that young horses may do more so than older horses, which may be associated with a learned response—you get problems.

On the other hand, muzzling seems to be very effective, which has been readily apparent in this 2002 season. This information has come back from the monitoring program, and we heard it again this morning from Dr. Riddle. So the bottom line is: if you can keep the caterpillars out of the mouth/intestinal tract of the horse, then we would seem to have gone a long way toward solving our problem.

When caterpillars do get into the oral cavity, what we pick up throughout the affected horses are bacteria that are apparently mouth commensals, the *Actinobacillus* and the non-hemolytic *Streptococcus* species. These bacteria, normally mouth residents, suddenly start to appear elsewhere in both pregnant and non-pregnant horses. They appear in the early and late fetal losses (EFL/LFL), and they also appear in the pericardial sac; we don't know what appears in the eye because we've not done bacteriology in the eye. Actinobacillus also appears in the brain: Drs. Sebastian and Harrison have reported three cases of Actinobacillus encephalitis occurring in or about the time of MRLS. When the caterpillars appear, something goes through *all* caterpillar-exposed horses, but it is in the pregnant mare that we see by far the most dramatic effects.

So, what happens when the caterpillars get into the horse's mouth? I like Dr. LeBlanc's analogy. Dentists working in my mouth put me on prophylactic antibiotics immediately because I have a heart murmur, and they don't want to risk a bacterial vegetative endocarditis. Likewise, something happens when horses are exposed to caterpillars in that we suddenly have oral commensal bacteria appearing shortly thereafter at multiple locations in the body. (In this regard, Dr. Sebastian has since drawn my attention to an un-referenced citation in an early edition of Blood and Henderson noting that mouth lesions in horses are associated with "hairy caterpillars" [1]).

We have been to some extent overwhelmed by the EFL and LFL—these are what has drawn attention to this whole problem—but there are also related things going on at a much lower rate in all Central Kentucky horses exposed to caterpillars.

Dr. Bernard isn't here, but I understand that he has shown that if you take a caterpillar and separate the exterior from the interior, the fetal losses appear to be associated with the integument, the outside of the caterpillar. This and other considerations drove the first mouse setal experiments that Dr. Sebastian has described to you. The setal hypothesis started with the thought that perhaps there was a toxin associated with the setae. Then we backed up and said that perhaps it's simply the setae themselves facilitating the movement of bacteria into the blood. We wondered about the setae themselves becoming little septic emboli in the body and carrying little quanta of infected material to various locations in the body. The infected material would be contaminated with bacterial commensals from the point at which the setal fragments entered the body of the horse. In MRLS cases occurring in the field, these would be the mouth commensals, the *Actinobacillus* and the non-hemolytic *Streptococcus* species. In experimental MRLS, where we delivered the caterpillars into the stomach by nasogastric tube, the bacterial picture is different, apparently consistent with the different point of entry for the bacteria.

Tissue localization of such septic emboli would not cause significant problems in most areas of the body, where the immune system can handle it, but some areas of the body may be particularly susceptible, such as the fetal membranes, and perhaps the eye, where the results of such effects are easily visible, and also the pericardial sac.

So, let me just simply say: Do we need a toxin? Well, we don't have a candidate toxin. Dr. Whitwell very kindly asked the toxicologists here to nominate a toxin, and one wasn't forthcoming. My sense at this time is that we need to look carefully at the link between the bacterial commensals in the mouth and how the outside of the caterpillars (and those barbed setae) may facilitate distribution of mouth commensals to distant locations in the body. At this point, I am far from persuaded that there's a classic toxicity mechanism involved, and I am a toxicologist, more or less, by training.

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Session 6

Experimental Replication of Mare Reproductive Loss Syndrome

Chairperson: Dr. Nancy Cox, Associate Dean for Research, College of Agriculture, University of Kentucky

Induction of Mare Reproductive Loss Syndrome by Directed Exposure of Susceptible Mares to Eastern Tent Caterpillar Larvae and Frass

B. A. Webb, W. E. Barney, D. L. Dahlman, C. Collins, N. M. Williams, and K. J. McDowell

RESEARCH INTO THE CAUSE OF MARE REPRODUCTIVE LOSS Syndrome (MRLS) has been ongoing since the disease was recognized in the spring of 2001. We report here results of a collaborative project involving the Department of Entomology, the Maxwell H. Gluck Equine Research Center, and the University of Kentucky Livestock Disease Diagnosis Center (UKLDDC). An intensive survey of horse farms in Central Kentucky in 2001 under the leadership of Dr. Roberta Dwyer found a strong correlation between the presence of eastern tent caterpillars (ETC) on farms and the incidence of MRLS. This project was designed to determine if the association was simply a *correlation* or if tent caterpillars were *causally* associated with MRLS. Therefore, these studies were also designed to mimic on-farm conditions by exposing pregnant mares to ETC in the fields on which the mares grazed.

Methods

Two experiments were performed. In Experiment 1, pregnant mares (between approximately 40 days and 7 months gestation) were exposed to increasingly high levels of ETC and their frass (n = 10 mares) or increasing levels of frass (n = 9 mares). In the control group (n = 10 mares), mares were handled identically to the experimental treatments, but attempts were made to minimize exposure to ETC larvae and frass. Treatments were administered in a randomized split plot design with animals subjected to experimental conditions in 16 x 16 ft pens in 20 x 200 ft treatment plots for 6 hours/day over two 10-day periods of exposure. The experiment was designed such that plot size was reduced with consequent increase of exposure over the course of the treatment. When not in pens, mares were pastured communally in adjacent areas. Mares were examined by manual palpation and ultrasonography once a week.

In Experiment 2, pregnant mares (between approximately 40 days and 6 months gestation) were divided into three treatment groups of eight mares each. Treatments were increasing levels of starved ETC, increasing levels of ETC frass, or control (no treatment added to the pasture plots). The ETC were collected two to three weeks prior to the experiment and had not been fed; thus, they would deliver little or no frass to the treatment plots. Additionally, ETC used in this study included mature, wandering-stage caterpillars. The ETC frass was collected two to four weeks prior to the experiment and was stored frozen. The design was to separate potential effects of the caterpillars themselves from those of the caterpillar frass. For 6 hours each day over a 10-day period, mares were placed individually in 16 x 16 ft pens, on plots as described for Experiment 1. Pasture plots for Experiment 2 did not overlap areas of the pasture previously used for Experiment 1. Turn-out fields for the mares when they were not in the experimental pens was the same for Experiments 1 and 2. All mares were examined daily, beginning prior to the onset of treatment and continuing for 10 days after the last treatment. Mares were then examined weekly for an additional four weeks.

Results and Discussion

In Experiment 1, pregnancy losses were 7 of 10 mares deliberately exposed to ETC and frass, 7 of 9 mares exposed to frass only, and 3 of 10 in the control group. Diagnostic evaluations of the mares and recovered fetuses were consistent with signs observed for fetal losses suffered in MRLS in 2001. In Experiment 2, pregnancy losses were 3 of 8 in the starved ETC group, 0 of 8 in the frass group, and 1 of 8 in the control group. Ultrasound image echotexture of the fetal fluids, as well as pathologic and bacteriologic findings, were consistent with MRLS-type abortions for all losses that occurred in both experiments.

These studies provided the first experimental evidence that ETC induce pregnancy loss in horses and were the first to reproduce the syndrome under experimental conditions. Furthermore, they showed that exposure to ETC and frass in Experiment 1 induced losses under conditions that mimicked field exposure. Experiment 1 was formulated with the objective of reproducing the syndrome in advance of its appearance in the field and thereby to provide timely information to farm managers and veterinarians in the region. Losses in this experiment were advanced relative to the occurrence of MRLS elsewhere in Central Kentucky in 2002, and preliminary results of the study were released in late April and reinforced recommendations to minimize exposure of susceptible mares to ETC. The four mares in the control groups that lost their pregnancies (three in Experiment 1 and the single loss in Experiment 2) were all in close proximity to one plot that

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received ETC as its experimental treatment. Monitoring of all pens for ETC indicated that the ETC containment system failed in some plots and resulted in elevated ETC levels in adjacent control plots. Mares in the control plots that suffered losses were all adjacent to ETC treatments and adjacent to the plot in which partial failure of the containment system was indicated. Therefore, it is highly likely that mares in these control treatments were exposed to ETC larvae in Experiment 1 with this exposure causing the observed losses. Based on these studies, we conclude that ETC larvae are *causally* associated with MRLS, but involvement of ETC frass remains unclear.

Gastric Administration of Eastern Tent Caterpillars Causes Early Fetal Loss in Pregnant Mares

B. Bernard, B. Webb, and M. LeBlanc

GASTRIC ADMINISTRATION OF STARVED EASTERN TENT CATerpillars (ETC) resulted in early pregnancy loss in the mare. The study involved three groups of mares. One group received starved caterpillars, one group received caterpillar frass, and one group served as control. Four of five mares administered caterpillars lost pregnancies within 8 to 13 days subsequent to the first dose of ETC. No control mares or mares that received frass aborted.

Materials and Methods

The study design consisted of three groups of five mares each. Mares were administered their respective treatment by nasogastric tube for 10 days. Group 1 mares served as controls and received 50 ml of water, Group 2 mares received 2.5 g of stored frass diluted in 50 ml water, and Group 3 mares received 50 g of crushed fresh ETC mixed in 50 ml of water. Mares were housed in stalls with no exposure to grass beginning 12 days before they were given any treatment and remained in stalls for the entire experimental period. Mares were walked twice daily to provide exercise. Mares were between 38 and 88 days of gestation on day one of treatment.

Results

Early fetal losses (EFL) were observed in 4 of 5 Group 2 mares. No control mares or mares receiving frass aborted. The mares that aborted were 49, 64, 70, and 96 days of gestation. *Alpha streptococcus* was cultured from one aborted fetus, while *Serratia* sp. was cultured from the remaining three fetuses. Neither pericarditis or ophthalmologic disease was observed. There were no significant changes in serum chemistries or leukograms. One of the aborting mares exhibited signs of abdominal pain two days prior to abortion.

Discussion

In the spring of 2001, a severe illness affecting pregnant mares occurred in Central Kentucky. The condition occurred across breeds and under a variety of management conditions. A variety of theories regarding the causative factor or factors has been proposed, one of which is the caterpillar or its frass. An epidemiologic survey identified a relationship between high numbers of ETC and EFL (1). A field study (B. Webb et al., this proceedings) suggested that ETC and/or frass were causally related to MRLS. This study confirms that ETC can cause EFL. The toxic agent (biological or chemical) is yet to be elucidated.

Conclusion

The investigators concluded from this study that ETC can cause EFL in the pregnant mares and that "stored" frass does not cause EFL. The study did not define the toxic component of the caterpillar responsible for the EFL.

Acknowledgments

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Experimentally Induced Mare Reproductive Loss Syndrome Late Fetal Losses with Eastern Tent Caterpillars

M. Sebastian, D. Williams, L. Harrison, J. Donahue, T. Seahorn, N. Slovis, D. Richter, T. Fuller, C. Trail, R. Douglas, and T. Tobin

DURING 2001, CENTRAL KENTUCKY HAD AN EPIDEMIC OF early and late fetal losses (EFL/LFL), which was together called Mare Reproductive Loss Syndrome (MRLS). The LFL began in the last week of April, peaked on May 5, and declined rapidly. EFL was identified on April 26 and had a similar course and ultimately totaled about 1,500 cases. A total of 450 LFL cases were submitted to the University of Kentucky Livestock Disease Diagnostic Center (UKLDDC) for detailed pathological examination during a period of two months (1). Concurrent with the epidemic was a local population explosion of eastern tent caterpillar (ETC), *Malacosoma americanum*.

Experiments conducted by Webb et al. (2) and Bernard et al. (3) have established a causal role for ETC in EFL. The necropsy examination of LFL demonstrated lesions not observed in EFL and bacteriological findings that can be compared to EFL. If reproduced experimentally, these observations will help in defining the specific pathogenesis, etiological agent, and clinical picture. Hence, a study was undertaken to evaluate the ability of ETC to reproduce abortion in late and midterm pregnancies.

Materials and Methods

Eleven pregnant mares obtained from a commercial nurse mare operation in late stage of pregnancy (9 to 11 months of pregnancy) were selected for the experiment. Mares were divided into two groups, six in the treatment group and five in the control group. Mares in the treatment group were administered 50 g of ETC collected from the Upper Peninsula of Michigan fed on wild cherry tree leaves mixed in 70 ml of normal saline for 9 days. The control group mares received 120 ml of normal saline for 9 days. All of the mares were confined to the stall during the entire period of experiment. They were fed hay and had constant access to clean drinking water. Mares were walked once daily to provide exercise. Blood was collected from the jugular vein 5 days prior to experiment and all 9 days of the experiment and every other day after the experiment for 10 days. Complete blood count and biochemical assays were performed within 2 to 3 hours of collection with an auto analyzer. Blood was collected aseptically from all the mares prior to experiment and also during the entire period of 9 days of experiment for bacterial culture. The serum concentration of progesterone and estrogen were estimated on the day before the experiment and on the day of abortion.

All the mares were monitored prior to the experiment by rectal ultrasonography and on days 3, 5, 7, and 9 by transabdominal ultrasonography for fetal heart rate, placental thickness, and appearance of fetal fluid.

Results

Five of the treatment mares aborted during the experiment, and the sixth mare aborted on day 15 from the first day of exposure. A complete necropsy was done on all aborted fetuses and placentas. A uterine biopsy was taken from all the aborted mares on the day of abortion. FATs were done on pooled tissue samples of all fetuses for *Leptospira* species and EHV. The first abortion occurred between 56 to 69 hours, and the last abortion at 357 hours on the fifteenth day. All the placentas had intact cervical stars, and two had tears of the allantochorion. The weight of the placentas ranged from 5 to 10 pounds. The length of the umbilical cords ranged from 55 to 82 cm. All the placentas' chorionic surface had a pale brown color compared to the dark red color seen on the chorionic surface of fresh placentas. The weight of the fetus ranged from 35 pounds to 82 pounds.

Enterobacter sakazakii (two fetuses), *Serratia marcescens*, and *Enterococcus* species (two fetuses) and *Enterobacter cloacae* (two fetuses) were isolated from multiple organs of the aborted fetuses. There was no significant difference in the complete blood count, serum biochemistry, coagulation factors, and ammonia levels between the treatment group and the control group. Detailed histopathological examination of the fetus and placenta showed hemorrhage and congestion of adrenals (6 of 6 fetuses), congestion of liver (5 of 6 fetuses), congestion of kidney (5 of 6 fetuses), hemorrhage in epicardium (3 of 6 fetuses), congestion of thymus (6 of 6 fetuses), congestion of spleen (5 of 6 fetuses), endometritis (6 of 6 mares), bronchopneumonia (1 of 6 fetuses), hepatic necrosis (1 of 6 fetuses), placentitis (1 of 6 fetuses), amnionitis (1 of 6 fetuses), and

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funisitis (1 of 6 fetuses). The fetal heart rate was increased in all fetuses on days prior to the day of abortion. FATs done on the pooled tissue samples of all aborted fetuses were negative for EHV and *Leptospira* species. Blood culture for bacteria yielded no bacteria during the entire period of experiment. There were no significant observations in the fetal fluid or placental thickness. All the fetuses showed significant increase in fetal heart rate before they aborted or were diagnosed dead *in utero*. The serum concentration of progesterone from the day before exposure of caterpillars, when compared to the day of abortion, had a drastic drop in concentration. The serum concentration of estrogen on the day of abortion was significantly lower than the day before the exposure of caterpillars.

Discussion

One consistent finding for all abortions was an intact cervical star similar to the gross finding in naturally occurring cases of LFL. Also, the chorionic surface of 4 of 6 allantochorions had a light brown yellow appearance, indicating placental detachment that remained *in utero* for sufficient time to cause fetal death. The ultrasound examination of the fetal heart rates showed an increase in heart rate indicating the fetuses may have been under stress due to hypoxia. Placenta detachment may have occurred leading to physiological effects consistent with fetal hypoxia. The fetus of the mare that aborted on day 15 had fetal diarrhea, which likewise underscores a possible connection to fetal hypoxia. Congestion and hemorrhage noted in fetal tissues may also be seen as indication that fetal hypoxia is a factor in the pathogenesis of MRLS. The fetus of the mare aborted on day 15 had fetal diarrhea and funisitis similar to the fetuses in naturally occurring cases of LFL (4). Enterobacter species, *Serratia marcencens*, and. Enterococcus species bacteria isolated in these cases were also isolated in naturally occurring cases of LFL (5).

The dose of ETC used for this experiment was based on the dose used in the EFL experiments (2,3). Abortion occurred earlier in this experiment as compared to the EFL experiment by Bernard et al. (3) in which the first abortion occurred on day 8. The first abortion in this study was observed at 69 hours post administration of ETC. The apparent difference in the early onset of abortion may be due to ETC in this experiment being fed fresh cherry tree leaves until the day of preparation, while in the EFL study by Bernard et al. (3), the ETC had been kept away from cherry tree leaves several days prior to preparation and administration. This difference in the onset of abortion/fetal death suggests that the caterpillars fed cherry tree leaves have more abortifacient potential when compared to starved caterpillars. Another fact to consider is that metabolically active ETC may be producing an abortifacient agent in much higher quantities compared to starved ETC.

Conclusion

This experiment indicates that dosing late-term pregnant mares with a preparation of ETC that had consumed wild cherry trees leaves causes abortion consistent with MRLS. Caterpillars were collected from the Upper Peninsula of Michigan and were shipped to Lexington for the experiment, indicating that ETC from other states have the potential to induce LFL in horses.

Acknowledgments

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Session 7

The Black Cherry Tree/Eastern Tent Caterpillar/ Eastern Tent Caterpillar Frass System

Chairperson: Dr. Lee Townsend, Department of Entomology, College of Agriculture, University of Kentucky

The Biology of the Tent Caterpillar As It Relates to Mare Reproductive Loss Syndrome

T. D. Fitzgerald

DURING THE SPRING OF 2001, HORSE FARMS IN CENTRAL Kentucky experienced an unprecedented loss of foals. Affected horses exhibited signs collectively referred to as Mare Reproductive Loss Syndrome (MRLS). Concurrent with the onset of MRLS was a population explosion of the eastern tent caterpillar (ETC), which drove starving caterpillars from the canopies of defoliated trees into adjacent pastures housing pregnant mares. Although the exact cause of MRLS remains unknown at this time, there is a growing consensus based on epidemiological surveys and preliminary experimentation that the caterpillar may have played a central role in the outbreak of MRLS. It is the purpose of this paper to outline the basic features of the biology and population dynamics of ETC as they relate to MRLS and to consider specific ways in which the caterpillar might cause harm to vertebrates. Unless otherwise referenced, the information presented here is derived from (1) and references therein.

Twenty-six species of tent caterpillars (*Malacosoma:* Lasiocampidae) occur in the northern latitudes of both the New and Old World. Six species are found in North America, but the only species to occur in Kentucky are the forest and ETC. The forest tent caterpillar (*M. disstria*) has the largest range of all the North American species, while the ETC (*M. americanum*) is largely limited to the eastern half of the United States and Canada. The two species are readily distinguished by larval color patterns, host choice, and behavior. *M. disstria* is the only species of tent caterpillar that does not construct a communal silk tent; sibling aggregates rest gregariously in the open on leaves and on the bark of the host tree.

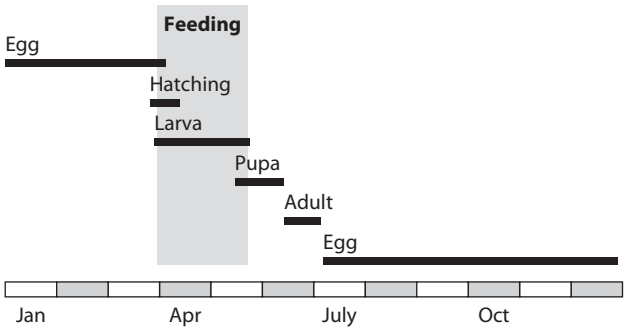
Local population of forest and ETC are found wherever their host species occur. The ETC is largely restricted to trees in the plant family Roseaceae, greatly preferring the black cherry but may also oviposit on choke cherry, fire cherry, apple, plum, peach, and pear, and more rarely on hawthorn, flowering quince, mountain ash, and *Cotoneaster*. Starved caterpillars and full-grown caterpillars that have dispersed from their natal tree may attempt to feed on other species of plants as well. The forest tent caterpillar may also feed on fruit trees, including black cherry, but prefers tupelo in the Southeast, sugar maple in the Northeast, and poplar in the North Central states and Canada. Because of its broad host range, which includes many important species of forest trees, the forest tent caterpillar is considered the most economically important of the North American tent caterpillars. However, it has thus far not been implicated in any naturally occurring instance of MRLS.

All species of tent caterpillars have similar life cycles (Figure 1). The overwintered eggs of the insect hatch in the early spring, just as the buds break and leaves begin to unfold, but it is not unusual for the caterpillars to emerge even before leaf flush and to subsist by mining the buds of the plant. The caterpillars are adapted to the chemistry of young leaves, placing a premium on early emergence.

ETC build a communal silk shelter (tent) that they expand daily to accommodate their growth. The caterpillars are central place foragers, and all activity is centered about the tent. When young, colonies launch forays to feeding sites distant from the tent in the afternoon, at dusk, and in the early morning before dawn. In the last instar, caterpillars from undisturbed colonies typically feed only at night, returning to their tent at dawn. The caterpillars are highly social and conduct all their activities in tight synchrony, moving to food, feeding, and returning to the tent en masse. After feeding, they rest together in or on the tent until their next foraging bout.

The caterpillars are ravenous feeders, completing their larval growth in as few as seven to eight weeks. Indeed, the ETC is among the fastest growing of all caterpillars. In the field, growth is constrained by low seasonal tempera-

Figure 1. Seasonal life history of the ETC in Kentucky. The exact time of egg hatching is temperature dependent and varies from year to year.



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tures, but in the laboratory at room temperature, the larvae reach their full size in only three weeks, increasing in mass during this period by a factor exceeding 5,000. When fully grown, the caterpillars drop from their natal tree, often traveling long distances over the ground in search of sheltered sites. Here the caterpillars spin cocoons and metamorphose, spending two to three weeks in the pupal stage before emerging as moths.

The adult moth is short lived and does not feed. The female emerges from the cocoon in late afternoon and secretes a sex pheromone to call males. Impregnated females typically oviposit that same evening. Thus, the female may emerge, mate, and oviposit in rapid succession, and being completely spent, die shortly thereafter.

The moth produces a single egg mass that contains approximately 300 eggs, but the exact number is highly variable. The eggs are wrapped around a branch of the tree near its tip and covered with spumaline, a frothy secretion from the female's accessory gland. The material serves to protect the eggs from parasitic wasps and desiccation.

Although embryogenesis occurs soon after the eggs are laid in June or July, the small larvae lie quiescent until the following spring. Thus, the most durable life stage of the insect is the small, inactive caterpillar that spends nearly nine months sequestered within the shell of its egg. For those concerned with managing populations of the insect, the caterpillar is most vulnerable during this stage of its life. The egg masses are readily seen after the leaves have fallen from trees in the autumn and can easily be cut from the branches and discarded. Indeed, this was a favored method of control in the early part of the twentieth century when entomologists approached the control of the caterpillar with a near missionary zeal. In some areas, contests were held and school children rewarded for every egg mass they collected.

Wherever they occur, ETC have boom-and-bust population dynamics; outbreaks alternate with periods of scarcity. Because of its short life cycle and the capacity of a single female to produce a large number of eggs, the reproductive potential of the ETC is enormous. In theory, a single pair of moths can produce a population of nearly 100 million caterpillars in only four seasons. Although local populations never fully achieve their full potential for exponential growth, they typically reach high densities for several years in succession, then collapse and all but disappear. Records collected for well over a century show that the duration of outbreaks and the interval between them is highly variable. The most complete records, complied for the forest tent caterpillar, indicate that outbreak populations may last for as little as one year to as many as nine.

The irruptive population dynamics of tent caterpillars are attributable to both density-dependent and density-independent mortality factors. The major density-dependent mortality factors are predation, disease, and starvation. The ETC has more than 200 known predators and parasitoids. They are often present in abundance during the late stages of outbreaks, and some have been credited with bringing population surges to an abrupt end. The caterpillar is also susceptible to viruses, bacteria, and microsporidians, particularly at high population densities.

When population densities peak, ETC commonly defoliate their natal trees well before they are fully grown, forcing them to strike off over the ground in search of a new food plant, an endeavor in which they are rarely successful. The caterpillars show great initial reluctance to leave their existing trail system and typically make little progress through the physically complex ground cover. Even after several days, colonies may succeed in traveling only a few meters from the natal tree. During these off-the-tree forays, the caterpillars initially maintain their social instinct, moving only short distances from their siblings and marking their progress with silk and pheromone pathways that others follow. The caterpillars have rudimentary eyes and can visualize the silhouettes of plant stems from short distances, and they may attempt to orient visually to them. It is likely but untested that when moving through pastures, the caterpillar may mistake the legs of horses for tree stems and orient to them as well. Regardless of their efforts, dispersing caterpillars rarely find another suitable host. They typically become isolated from their siblings and marooned on unpalatable plants where they eventually starve or are killed by predators.

Inclement weather is the preeminent density-independent mortality factor affecting populations of tent caterpillars. This is the case because the caterpillars cannot process food when their body temperatures fall below 15°C. To achieve the body temperatures needed to digest food, the caterpillars bask in the sun on cold spring days, either on or in their tent. Their black bodies absorb solar radiation, and the protective layers of the tent minimize convective heat loss. If the days are cold and cloudy, the caterpillars grow little or not at all, and if these conditions persist for two weeks or more, colonies begin to perish. Occasionally, late-season frost kills the leaves of the host tree, and the caterpillar populations, left without food, experience a region-wide die-off. Many instances of population collapses due to cold springs have been documented in the northern part of the ranges of both the forest and ETC.

When a tentative association between the ETC and MRLS was first made in 2001, the caterpillars were near the peak of their population cycle in many of the affected areas. Reports from the field indicated that pastures were overrun with dispersing caterpillars. Masses of caterpillars were observed walking along the rails of fences, and many fell into troughs used to water horses. Thus, it became of urgent interest to explore all aspects of the biology of the

caterpillar in an attempt to determine what attribute of the insect might link it to MRLS (Table 1). One of the first hypotheses to be to tested was the possibility that the caterpillars had poisoned pregnant mares with cyanide, triggering abortions and stillbirths. The principal host of the ETC, the black cherry, is capable of producing cyanide in response to herbivory. The leaves of the tree contain the cyanogenic glucoside, prunasin. When ingested by ETC, or any other herbivore, enzymes in the leaf convert prunasin to mandelonitrile, then to benzaldehyde and cyanide. A recent study showed that at the time of the year when ETC feed on cherry trees, the leaves have an average cyanide potential (HCN-p) of 1,900 ppm (2). Young leaves at the tips of branches have a mean HCN-p of more than 3,000 ppm, while oldest leaves at base of the stems have an HCN-p of about 1,100 ppm. ETC prefer the youngest and most cyanogenic leaves. When allowed to feed overnight on young leaves, the bolus of the foregut contained an average about 600 ppm cyanide the next morning, while the midgut bolus contained an average of only 14 ppm (2). The caterpillars are unable to digest much of the leaf, and approximately 50% of the energy of the leaf is egested as fecal pellets. When dry, the pellets contain less than 100 ppm of cyanide. Thus, the ETC quickly detoxifies cyanide, and compared to the amount of cyanide that an herbivore might ingest if it fed on cherry leaves, it would acquire relatively little cyanide if it inadvertently consumed ETC or their fecal pellets. Most damaging to the cyanide hypothesis was the result of another study showing that when pregnant mares were treated with higher concentrations of cyanide than they could reasonably be expected to assimilate by ingesting caterpillars or their fecal pellets, they exhibited no signs of MRLS (3).

Another attribute of ETC having the potential to cause harm to animals that come in contact with them is its hairs or "setae." Some caterpillars, such as those of the buck moth (*Hemileuca maia*), have poisonous hairs that sting the victim when touched. These "urticating" hairs act like hypodermic needles, bearing a sharp point that penetrates the skin and a hollow shaft filled with poison. The most notorious of the urticating caterpillars is the pine processionary of southern Europe (*Thaumetopoea pityocampa*), whose larvae form long, head-to-tail processions as they move over the ground in search of pupation sites. Compared to the buckmoth, the hairs of the pine processionary are lighter and more brittle, scatter easily in the wind, and can affect individuals who have no direct contact with the caterpillars. It was recently discovered that susceptible individuals are also capable of exhibiting an immunologic response to a setal protein carried by the processionary, and instances of anaphylaxis have been reported (4). In contrast to the setae of the caterpillars of the buckmoth and pine processionary, the soft setae of the ETC are not designed to penetrate skin, and they are nonpoisonous. Yet, like the hairs of the processionary, they appear capable of causing an allergic skin response in individuals who handle large numbers of them over extended periods, but there have been no scientific studies to document these effects.

Table 1. Attributes of ETC potentially capable of harming vertebrates.

	Pathway		
Attribute	**Ingestion**	**Inhalation**	**Mode of Action**
cyanide	x[1]		toxin
calcium oxalate	x[1]		toxin
cuticular ketosteroids	x[1]		potential hormone mimic/anti-hormone[3]
setae/crochets	x		mechanical/chemical abrasion of mucous membranes
setae/setal fragments	x[2]	x	antigen/immunologic
pathogen vector	x[2]	x	infection

1 Large numbers of caterpillars may need to be ingested.
2 May be ingested along with grass or other forage passively contaminated by dispersing caterpillars.
3 Uninvestigated possibility.

The last instar of the ETC accumulates calcium oxalate in its Malpighian tubules, structures that are the analogs of the kidneys of vertebrates. The ingestion of calcium oxalate can cause inflammation of the lining of the stomach and intestines, but it is not known if the small amount of the material found in the Malpighian tubules of the tent caterpillar has any significant effect on animals that ingest them. The caterpillar adds the oxalate to its cocoon while spinning, and it serves to stiffen it. If the cocoon is disturbed, the oxalate billows up in a yellow cloud and is thought to act as a deterrent to would-be predators. Anecdotal reports indicate that inhalation of the powder can irritate the respiratory tract, and contact with mucous membranes or skin may cause symptoms of redness, swelling, itching, and pain in susceptible individuals, but there have been no definitive studies to document any of these effects.

The ETC stands at the pinnacle of sociality among caterpillars. Caterpillars explore the branches of the host tree in a search for food. Successful foragers mark their pathways back to the tent with a recruitment pheromone that serves to guide other caterpillars to their food-find. Two pheromone components have been identified, and both are ketosteroids. There is the uninvestigated possibility that these compounds may have hormonal or anti-hormonal activity when consumed by predators. But because they occur in nanogram quantities, it would seem unlikely that they would have a significant impact on an animal as massive as a horse.

There is also the possibility that if a horse ingests a caterpillar, some attribute of the caterpillar may facilitate the

invasion of potential pathogens that ordinarily reside harmlessly in the digestive tract of the horse. The setae or the sharply pointed crotchets of the prolegs could irritate the mucous membranes of the gastrointestinal tract of a vertebrate predator and create an infection court. A toxin produced by the caterpillar might act in much the same way.

Although no species of caterpillar is presently known to serve as a vector of a vertebrate pathogen, nor has any pathogen been thus far implicated in MRLS, a reasonable working hypothesis is that the ETC serves as a vector for a microbe that is a causal agent of MRLS. A good model species for such a pathogen is the bacterium *Serratia marcescens*, both because it has been isolated from the gut of field-collected caterpillars (5) and because it is capable of causing serious infection in horses (6,7). The bacterium has been little studied in tent caterpillars, but investigations of the tobacco budworm (8) indicate that it can be transferred from one generation of the caterpillar to the next on or in the egg of the moth. Moreover, the bacterium responds by multiplying rapidly when host caterpillars are stressed. The bacterium is typically benign in the gut of caterpillars, but if it gains access to the hemocoel, it causes rapid death (5). In species that can be killed by ingestion of the bacterium, oral doses of from 10^5 to 10^6 bacterial cells are typically required to produce significant mortality, indicating that common strains have low virulency. More virulent strains of *S. marcescens*, however, have been isolated. Farrar et al. (9) studied a strain that caused mortality in excess of 70% when fewer than 100 bacterial cells were fed to the corn earworm. Thus, studies of *S. marcescens* show that particularly virulent strains of viruses or bacteria may arise from time to time within populations of caterpillars and multiply rapidly when the insects are at peak population levels and stressed by starvation. Because of their enhanced virulence and abundance, these biological agents might pose a particular threat to cross-sensitive animals that contact the dispersing insects or their contaminated fecal pellets. Moreover, if the presence of virulent biological agents in populations of ETC is both a temporally and spatially transient phenomenon, the co-occurrence of ETC and pregnant mares need not invariably result in reproductive losses.

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Age-Specific Patterns of Eastern Tent Caterpillar Dispersal: Implications for Reducing Mare Reproductive Loss Syndrome through Population Management

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THE EASTERN TENT CATERPILLAR (ETC), *MALACOSOMA americanum* (Fabricius) (Lepidoptera: Lasiocampidae), has been implicated in Mare Reproductive Loss Syndrome (MRLS) (1). This native defoliating insect overwinters as eggs in bands encircling small diameter twigs, and initial egg hatch coincides with bud break in early spring. Caterpillars feed gregariously and prefer wild cherry foliage (2), which grows abundantly in Kentucky along pastures and roadsides. Larvae construct conspicuous silken tents that increase in size as the caterpillars mature (2,3). At endemic population levels, caterpillars forage only as far as necessary to feed (4,5). But as populations increase to outbreak levels and preferred hosts are defoliated, host plant requirements become less stringent (6,7), and caterpillars are forced to forage greater distances to obtain food, thereby increasing the potential for exposure to grazing horses. ETC dispersal behavior has been studied extensively in the context of diurnal feeding patterns (4,5), trail pheromone production (8), and recruitment behavior (9), but nothing is known about how caterpillars disperse in pasture situations, nor how their dispersal behavior might be manipulated to affect population suppression.

Grazing restrictions, a limited treatment window, the potential for inclement spraying conditions, and environmental concerns make widespread insecticide applications for tent caterpillar control impractical on horse farms (10,11). Highly mobile, dispersing caterpillars and use of alternative trees by feeding caterpillars make efforts at focused control difficult. The ability to predict population behavior and develop more precise management strategies will be enhanced by a more complete understanding of age-specific caterpillar dispersal behavior and potential.

Caterpillar dispersal behavior was studied as it pertains to pasture situations in the Bluegrass region. The goal was to investigate the dispersal patterns of ETC from defoliated fence rows to potential alternative hosts and to potential overwintering sites. Specific objectives were to assess the dispersal patterns of foraging third and fourth instar ETC and compare them to dispersal patterns of wandering (late instar) caterpillars, with respect to dispersal direction, dispersal distance, response of caterpillars to visual stimuli, physiological capacity of caterpillars to disperse, and determining if the time of day influenced caterpillar dispersal behavior.

Methods

Experiments were conducted in the spring of 2002 using field-collected ETC that were collected by clipping intact tents from wild cherry trees in and around Lexington, Kentucky. As they matured and local sources were depleted, additional caterpillars were obtained from south-central Wisconsin. Caterpillars were held in the laboratory in growth chambers (23°C, 15:8 L:D), fed fresh wild cherry foliage as needed, and were starved for 12 hours prior to use. All caterpillars were sorted and aged (12), and only those that appeared healthy were used in assays. The study site was a ~1.5 hectare, roughly rectangular asphalt parking lot on the University of Kentucky campus.

Dispersal Distance

Caterpillars (n = 75) were released from the center point of the study site at 6 a.m. and 6 p.m. on three consecutive days (15 to 17 May 2002). There were three replicates of the 6 a.m. release, but because of inclement weather, there were only two completed replicates of the 6 p.m. release. Prior to their release, caterpillars were dyed with a fluorescent powder to facilitate tracking their movements. Caterpillar dispersal distance was monitored at 30, 60, 90, 120, and 360 minutes for those released at 6 a.m. and at 30, 60, 90, and 120 minutes for those released at 6 p.m. Evening releases could only be tracked for 2 hours because of darkness.

Dispersal Direction

To assess caterpillar dispersal direction, arenas consisting of 20 m diameter circles were drawn in chalk on the asphalt. There were four blocks, each containing four circular 20 m arenas, separated by a minimum of 10 m, for a total of 16 arenas. Caterpillars (n = 25) were released from the center of each arena, and dispersal direction was monitored for 120 minutes. When caterpillars reached the edge of the circular arena, they were removed from the study. Measurements were made of the final bearing from the release point for caterpillars that did not leave their arenas.

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Response to Visual Stimuli

Caterpillar reliance on visual stimuli for orientation was assessed in two ways. First, caterpillar stemmata were blocked with acrylic artists' paint, effectively reducing all visual stimuli. The altered caterpillars (n = 25) were then released in the center of each circular arena (n = 16), and dispersal direction was monitored for 60 minutes. Secondly, response to visual cues was assessed by measuring the orientation of sighted caterpillars to vertically oriented objects, designed to simulate tree-trunk images. Each circular arena contained vertically oriented objects consisting of two adjacent 122 cm sections of 25 cm diameter PVC pipes, one of which was painted black and the other of which was painted white, placed at the edge of the arena in a randomly assigned direction. Caterpillars (n = 25) were then released in the center, and orientation and movement was monitored for 60 minutes. Again, when caterpillars reached the edge of the circular arena, they were removed from the study.

Physiological Capacity of Dispersing Caterpillars

To assess the physiological ability of ETC to disperse, caterpillar stamina was tested on a treadmill. A small branch of host foliage, placed just out of reach of the experimental caterpillars, provided stimulus. Caterpillars were weighed prior to each trial, then placed individually on the treadmill (Model 1010-M3, Columbus Instruments, Columbus, OH) for 15, 30, and 120 minutes (n = 3), or for 240 minutes (n = 1). Preliminary experiments showed that caterpillars were easily able to travel at a rate of 2.7 m/minute. Treadmill speed was set at that rate for all replicates except the single replicate lasting 240 minutes, during which the speed was reduced for some portions of the trial. Following each trial, the caterpillar was immediately frozen and stored at -80°C for future lipid analysis. Distance traveled was then calculated.

Data Analysis

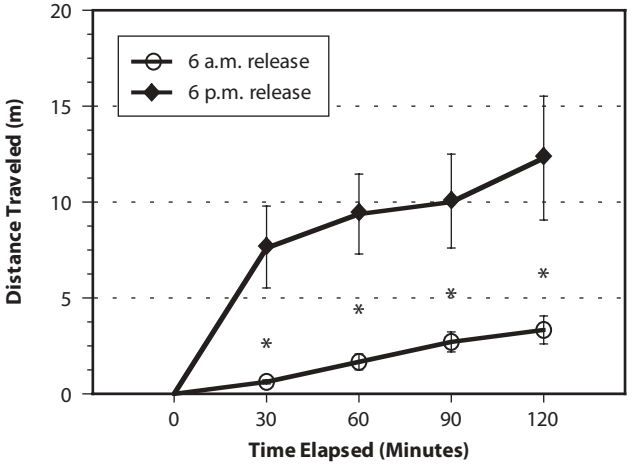
Analysis of variance was used to determine if distance traveled by released caterpillars differed between time increments, and linear regression was used to assess the relationship between distance traveled and time elapsed following release. After an analysis across all releases, a separate analysis for morning and evening releases was performed. Dispersal distance at each time increment (up to 120 minutes) for morning and evening releases was compared using a t-test. To assess the extent to which caterpillar dispersal direction was random, a chi-square analysis was performed separately for foraging and wandering caterpillars, for time of day (morning versus evening release), and for dispersal direction based on time elapsed (2 hours versus 6 hours post-release). To measure the randomness of orientation of caterpillars with blocked stemmata, a chi-square analysis was performed to assess the reliance of dispersing caterpillars on visual stimuli. Finally, a paired t-test was performed to assess differences in sighted caterpillar response to black and white vertically oriented objects.

Results

Dispersal Distance

Distance traveled by released caterpillars increased steadily and significantly ($F = 23.7$, $P < 0.001$), and was strongly correlated with the time elapsed in both the morning ($F = 13.4$, $P = 0.003$) and evening ($F = 11.1$, $P = 0.001$) (Figure 1) (F is a variance ratio test). Caterpillars released in the morning traveled at a fairly slow and steady rate of ~0.025 m/minute, equivalent to about 1.2 inches per minute, and covered an average distance of 8.4 m in 120 minutes. In the morning, the distance caterpillars traveled at 30 minutes differed from the distance traveled at 90 minutes ($P = 0.02$) and 120 minutes ($P = 0.003$), and the distance traveled at 60 minutes differed from that at 120 minutes ($P = 0.03$). However, there was no difference in distance traveled between the 30-minute and 60-minute time increments, between the 60- and 90-minute increments, and between the 90- and 120-minute increments. In contrast, the distance traveled by caterpillars released in the evening increased steadily but was similar across intervals. Caterpillars released in the evening traveled at a relatively rapid average rate of 0.17 m/minute (6.3 inches per minute) and covered an average distance of 39.3 m in 120 minutes, more than 4.5 times the distance of those released in the morning. The distance traveled by caterpillars released in the evening was greater than the distance traveled by caterpillars released in the morning at each time interval (Figure 1).

Figure 1. Distance travelled over time by ETC released from a central release point. Asterisks indicate significant differences between morning and evening releases at specified time intervals.



Dispersal Direction

ETC dispersal direction was non-random and biased away from the south (Table 1a). This was particularly evident with foraging caterpillars, whose directional movement was strongly non-random and was greatest toward the northwest, west, and north. Dispersal direction of the older wandering caterpillars was random and differed significantly from directional patterns of the younger foraging caterpillars (χ^2 = 47.3, P <0.0001).

The non-random dispersal pattern, biased away from the south, was more apparent in the morning (Table 1b), with caterpillar movement in the evening releases being only slightly non-random. The bias away from southerly movement persisted for up to 6 hours after the morning releases (Table 1c).

Response to Visual Stimuli

Non-random dispersal persisted even when caterpillar stemmata were blocked, which effectively eliminated visual stimuli. However, the pattern differed significantly from sighted caterpillars (χ^2 = 131.9, P <0.0001). In altered caterpillars, the bias in caterpillar orientation shifted, with the lowest response in the northwesterly direction (Table 1d).

Sighted caterpillars were responsive to vertically oriented objects, and when given a choice, showed a strong preference for black objects over white (Table 2).

Physiological Capacity of Dispersing Caterpillars

Caterpillars readily moved on the treadmill for 15-, 30-, and 120-minute intervals, traveling distances of up to 324 m (Table 3). The single caterpillar that lasted 240 minutes required some light prodding and eventually stopped from exhaustion after traveling a distance of approximately 624 m.

Discussion

During the morning hours, foraging ETC do not disperse randomly but show a bias away from southerly movement that is maintained for up to 6 hours following release. In contrast, foraging caterpillars released in the evening were more random in their dispersal direction, moving in all directions somewhat evenly. The magnitude of the differences in distance traveled between the morning and evening releases is striking since both releases approximate periods of tent caterpillar feeding activity (5). Caterpillar dispersal rate is temperature dependent and is a function of body length and body temperature (13). The air temperature at 6 a.m. was 12.3°C, with an average of 15.2°C over the 120-minute morning assay. At 6 p.m., the air temperature was 17.1°C, averaging 17.9°C over the corresponding 120 minutes. Although surface temperature measurements were not consistently taken over the course of the study, 6 a.m. surface temperature averaged 19.5°C over two measurements, and 6 p.m. surface temperatures were 27°C, also averaged over two measurements. Clearly the elevated evening temperatures could influence caterpillar dispersal rate.

Dependence of ETC on visual stimuli for orientation is evidenced by the shift in caterpillar movement when visual cues are obstructed. Polarized light, detected through caterpillar stemmata, may be used for orientation. Wellington (14) demonstrated that by rotating the plane of polarized light with polarizing filters a specified amount, tent caterpillar movement can be shifted by a similar amount. The shift in caterpillar movement when stemmata are obstructed supports the idea that polarized light may serve as a cue for caterpillar orientation. Caterpillar stemmata also detect light and allegedly are capable of crude image formation (15). Larvae swing their heads back and forth, scanning their surroundings, and can detect shapes and object orientation (16,17). Studies of other tree-feeding lepidopterans have demonstrated caterpillar orientation toward objects (18) and a limited ability to discern an object's size and relative distance (19). The results corroborate these findings and demonstrate that the ETC is able to detect the contrast between black and white and may be capable of limited color vision.

Table 1. Dispersal direction (% response) of ETC released under various conditions from the center of 20 m diameter circular arenas (n = 16).

		Compass Direction								
		N	NE	E	SE	S	SW	W	NW	χ^2 / *P*
a.	Caterpillar age									
	all caterpillars	20	16	13	8	8	8	14	14	42.2/ <0.001
	foraging	18	11	11	3	4	6	19	28	302.4/ <0.0001
	wandering	16	15	14	10	10	9	11	16	11.6/ 0.11
b.	Release time									
	morning	20	19	14	7	6	7	12	16	43.7/ <0.001
	evening	19	11	12	10	12	9	17	11	12.2/ 0.09
c.	Time elapsed									
	2 h post-release	19	13	13	9	9	9	15	14	19.7/ 0.006
	6 h post-release	21	22	13	6	6	6	11	13	32.9/ <0.001
d.	Visual status									
	sighted 20		16	13	8	8	8	14	14	42.2/ <0.001
	altered	12	14	16	13	14	13	11	7	26.8/ 0.004

[a] Caterpillar age: "foraging" are third and fourth instar caterpillars, and "wandering" are fifth and sixth instar caterpillars, independent of release time and time elapsed.

[b] Release time: 6 a.m. and 6 p.m., independent of caterpillar age and time elapsed.

[c] Time elapsed: 2-hour and 6-hour post-release, for morning releases only, independent of caterpillar age.

[d] Visual status: "sighted" are caterpillars with complete vision, and "altered" are caterpillars with stemmata obstructed.

Table 2. Response of foraging ETC to black and white vertically oriented objects.

Color	Mean (s.e.)
black	1.7 (0.2)
white	0.5 (0.1)
paired t-test / P	5.3/ <0.0001

Table 3. Distance traveled by foraging ETC in treadmill studies.

Rate (m/minute)	Time Elapsed (minutes)	Distance Traveled (m)	n
2.7	15	40.5	3
2.7	30	81	3
2.7	120	324	3
2.5-2.7	240[a]	624	1

[a] to exhaustion.

Future work on ETC dispersal as it pertains to MRLS will concentrate on developing a model to predict caterpillar location (distance and direction) based on time of day (morning versus evening) and time elapsed since caterpillar movement began. Preliminary work using a logit model (20) demonstrated that caterpillar distance and direction can be predicted based on time of day and time elapsed, but the existing model lacks precision.

Knowledge of caterpillar dispersal patterns will increase our ability to manage ETC populations and predict risks to specific pastures. In addition, this knowledge will help in the development of management protocol, such as isolation distances of mares from host trees, that will allow horse farm managers to more effectively reduce mare exposure to tent caterpillars, thereby reducing the risk of MRLS.

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Timing of Emergence of Eastern Tent Caterpillars and Management with Reduced Risk Insecticides and Treatment Strategies

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FARM SURVEYS (1, THIS PROCEEDINGS) AND EXPERIMENTAL induction of Mare Reproductive Loss Syndrome (MRLS) by directed exposure of susceptible mares to eastern tent caterpillar (ETC), *Malacosoma americanum*, in field plots (2, this proceedings) or by gastric administration (3, this proceedings) point to ETC being involved with the disease. This mandates that farms minimize exposure of pregnant mares to ETC at least until the mechanism by which the caterpillars are involved can be determined. Spraying tall trees bordering pastures with traditional insecticides is potentially hazardous to farm workers, horses, and the environment. Moreover, relatively few insecticides are labeled for pasture usage, and many products that are effective against ETC have grazing restrictions on their label. Issues of spray drift and liability thus complicate control options.

Reduced-risk insecticides and treatment strategies for managing ETC on horse farms were evaluated. The work emphasized target-selective compounds with low vertebrate toxicity, or micro-injection of trees to avoid spray drift. ETC egg hatch was monitored to help guide the timing of control actions. The 2002 results of these ongoing studies are summarized.

Materials and Methods

Timing of Emergence in Trees

Emergence of ETC from egg masses and subsequent colony development were monitored at three field sites, the University of Kentucky's Coldstream and South Research Farms and Gainesway Farm, near Lexington, Kentucky, in the spring of 2002. Sites were rows of mature wild cherry trees, *Prunus* spp., bordering pastures or fences. About 200 total twigs bearing egg masses were tagged with flagging tape from 11 to 19 February. Egg masses were checked every 1 to 2 days until mid-April, when all larvae had emerged. Larval behavior (e.g., aggregation on egg masses, movement to twigs, size of nests) and instars predominating were noted. Observations continued until larvae began wandering before pupation.

Twenty additional cherry twigs with single ETC egg masses were field-collected and kept outdoors in florists' water picks to maintain shoot turgor. These egg masses were monitored daily; upon eclosion, larvae were counted and removed. The pattern and duration of emergence from individual egg masses were determined, as well as the number of ETC per mass.

Horticultural Oil or Insecticidal Soap against Newly Hatched Larvae

Insecticidal soaps and oils seemingly were good candidates for ETC management because they are essentially nontoxic to vertebrates. Egg masses with newly emerged and soon-to-emerge larvae were collected from field sites. Twigs bearing egg masses with aggregations of newly enclosed larvae were sprayed to runoff with either a 3% solution of Superior miscible oil (Universal Cooperative, Minneapolis, MN), insecticidal soap (M-pede®, Mycogen, San Diego, CA) at labeled rate (31.3 ml/liter [4 fl oz/gal.]), or distilled water. Another set of controls was not sprayed. After treatment the larvae (about 25 per egg mass) were brushed off the twigs onto moist filter paper in separate petri dishes for each mass. Mortality was evaluated after 1, 4, 8, 24, and 48 hours. Data were adjusted for control mortality by Abbot's formula (5).

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Foliar Sprays for Controlling Early or Late Instars

Four insecticides with low mammalian toxicity were tested against young (first and second instar) or midsized (third and fourth instar) ETC feeding on fresh cherry foliage. These products were: *Bacillus thuringiensis* [Bt] (Dipel Pro®, Valent, Richardson, TX), a microbial insecticide active against many caterpillar species; Spinosad (SpinTor® 2SC; Dow Agrosciences, Indianapolis, IN), derived from fermentation of a naturally occurring soil bacterium (4); tebufenozide (Confirm® T/O, Dow AgroSciences, Indianapolis, IN), a molt-accelerating compound (ecdysone agonist); and bifenthrin (Talstar®, FMC, Philadelphia, PA), a pyrethroid. Each product was applied at label rate: Dipel at 2.64 ml/liter [34 fl oz/100 gal.]; SpinTor at 0.66 ml/liter [8 fl oz/100 gal.], Confirm at 1.32 ml/liter [16 fl oz/100 gal.], and Talstar at 0.84 ml/liter [10.8 fl oz/100 gal.]. A spreader/sticker (Breakthru®) was added to the tank mix at 0.31 ml/liter [4 fl oz/100 gal.] for all spray tests.

Tests with early instars were done twice, with woody shoots bearing either green-tip buds or expanded leaves, to simulate conditions encountered at emergence. Tests with larger larvae used fully expanded leaves. Larvae and shoots were field-collected from wild cherry trees. Shoots were trimmed to 25 cm length, placed temporarily in water-filled flasks, sprayed to runoff, with residues allowed to dry outdoors. Separate controls were sprayed with the spreader/sticker only or were not treated. Individual shoots then were inserted through a white 10-cm filter paper and the slotted lid of a water-filled plastic cup. A vented, acrylic cylinder was placed over each shoot to form a cage. The paper floor facilitated counting of moribund or dead larvae, as well as collection of frass. Treatments were replicated five times in each trial. Treated shoots were challenged with 20 ETC for tests with early instars or with 10 ETC for tests with third and fourth instars. Caged shoots were held in a growth chamber set to simulate concurrent field conditions: 9:15 (L:D) photoperiod, and 25°C or 15°C, respectively, during those cycles. Mortality of early instars was evaluated after 1, 8, and 24 hours, and daily thereafter for 7 days. Mortality of midsized ETC was determined daily for 7 days. Accumulated frass was collected, dried, and weighed.

Toxicity of Weathered Residues on Foliage

Persistence of the aforementioned insecticides was tested as follows. Pre-tagged cherry shoots were sprayed on 14 April, with residues allowed to weather in the field. Separate sets of shoots were harvested at 1, 3, or 7 days after treatment and challenged with third and fourth instars as above. Larval mortality was assessed daily for 4 days after each respective harvest.

Test for Behavioral Avoidance of Residues

It was important to determine if residues of the aforementioned insecticides might repel ETC because such response might cause larvae to avoid feeding on treated leaves or even to prematurely abandon treated trees and disperse into pastures. Shoots with fully expanded cherry leaves were treated on 30 April with residues allowed to dry. Treated leaves were paired with nontreated ones in 10-cm petri dishes on moist filter paper. There were 30 replicates for each insecticide. One fourth-instar ETC was added to each dish and left for 3 hours to take its first sustained meal (typically 10 to 20% of one leaf was consumed). Leaves then were electronically scanned, and missing leaf areas were compared.

Micro-Injection of Cherry Trees with Dicrotophos (Bidrin)

Mature cherry trees growing along a fence at Glenwood Farm, Versailles, Kentucky, were used in this study. The trees were heavily infested with many small tents containing mostly second-instar ETC. The trial was done on 10 trees in a continuous row. On 16 April, alternating trees were injected with dicrotophos (Bidrin, as Inject-A-Cide® "B") using the Mauget Micro-Injection System (Mauget, Los Angeles, CA). Dosage was determined by dividing tree circumference (in cm) at 1.25 m above ground by 15 (e.g., a 22.5-cm diameter tree received five capsules). Injections were done in the root flare, about 15 cm above ground, and evenly spaced around the tree. Control trees were not injected. Efficacy was determined after one week (23 April) by harvesting 10 nests per tree (50 nests per treatment) with a pole pruner or by climbing and sealing them in individual bags. Most of the sampled nests were 4 to 8 m above ground. Accurate whole-tree nest counts were impossible because the trees were flowering, and small tents could not be seen against the backdrop of white flowers. Each control tree was injected immediately following the 23 April sampling. Those trees were sampled again, in similar manner, on 30 April.

Bagged nests were taken to the laboratory where they were dissected and the caterpillars examined. Nests initially were scored: 1 = all larvae dead, 2 = a few (≤ 5 live larvae), 3 = 5 to 10 live larvae, 4 = 25 to 50 live larvae, 5 = robust, full nest. All 50 nests from the treated trees were then dissected, their larvae individually examined and counted. Nests from control trees all were robust, and many contained hundreds of live larvae. For controls, a representative sample (10 nests; two from each tree) was dissected as above.

Test for Residual Control of Wandering Larvae in Pastures

This trial was conducted in a mixed bluegrass pasture at the University of Kentucky's Spindletop Research Farm, near Lexington. Individual plots (6.4 x 6.4 m) were arranged in a randomized complete block with four replicates. Treatments were Malathion 25% EC at 1.4 kg AI/ha [1.25 lb AI/A], Sevin 4F at 1.12 kg AI/ha [1 lb AI/A], or untreated controls. Plots were sprayed on 7 May. When residues had dried, two rings of polyvinylchloride (PVC) pipe (15.2 cm high, 20.3 cm diameter) were seated about 1 m apart near the center of each plot. Ten fifth-instar ETC, collected from leaves of wild cherry trees, were placed inside each ring, the top 2.5 cm of which was greased with petroleum jelly to discourage escape. Plots were examined on May 8 and 10, and numbers of live and dead larvae were determined.

Preliminary Test for Residual Toxicity of Bifenthrin to ETC on Tree Trunks

The final test, done 14 May, examined whether dry residues of bifenthrin (Talstar) or permethrin (Astro®, FMC, Philadelphia, PA) on cherry bark might intercept and kill ETC crawling down tree trunks to disperse or pupate. Fifteen freshly cut sections of cherry trunk (2 m long, 15 to 20 cm diameter) were obtained from a tree care firm. These were supported vertically, out of doors, and treated with either Talstar (7.9 ml/liter [1 fl oz/gal.], Astro (13.2 ml/liter [1.7 fl oz/gal.), or not treated. When residues had dried, 10 late-instar ETC were placed on the top of each bolt, and their behavior was observed. Two separate groups of larvae were tested with each bolt.

Results and Discussion

Timing of Emergence in Trees

First observed emergence from egg masses was 15 March 2002, coincident with about 50% bloom of *Forsythia*. Once an egg mass became active, neonates continued to emerge for two to three weeks (mean = 17.9 ± 1.0 day, range 12 to 26 days). Area-wide emergence was slow during the first 1.5 weeks but accelerated as mean daily temperatures climbed. Emergence peaked from 29 March to 2 April and was nearly finished by 15 April. Only 44% of the egg masses had become active by 28 March, whereas 88% had done so by 3 April. Larvae did not begin to emerge from the last egg mass until 18 April. Small tents were visible in cherry trees by 1 April, and by 10 April many larvae were abandoning smaller nests on the tree periphery to join together into larger central nests in main tree crotches.

Horticultural Oil or Insecticidal Soap against Newly Hatched Larvae

Point-blank sprays with horticultural oil or insecticidal soap gave poor control of neonate ETC. Adjusted percentage mortality from those treatments averaged 27.2 ± 6.7 versus 38.1 ± 3.1, respectively, after 24 hours, and 37.1 ± 4.7 versus 49.1 ± 5.2, respectively, after 48 hours. Given the prolonged emergence period of ETC and the fact that oils and soaps kill by contact and have no residual activity, these products would have to be sprayed repeatedly, every few days, to get even partial control. Efficacy doubtless would be even less once ETC had molted to second instars. Oils or soaps would be further compromised by the difficulty of getting adequate coverage in tree canopies.

Foliar Sprays for Controlling Early or Late Instars

Dry residues of Talstar or SpinTor gave excellent control of first instars (100 and >95%, respectively, within 24 hours) on both green-tip buds and young leaves. Dipel also was effective but slower. Mortality of neonates gnawing on Dipel-treated green-tip buds was 31, 52, and 61% after 24, 48, and 72 hours, respectively. Corresponding levels of control on Dipel-treated expanding leaves were 43, 89, and 92%. Confirm, the molt-accelerating compound, worked more slowly than Dipel, providing only 45% control after 3 days, and about 75% control after 5 days. Talstar works both by contact and ingestion, whereas Spintor, Dipel, and Confirm must be consumed.

Against third and fourth instars, Talstar and Spintor gave 78 and 76% control, respectively, after 1 day; 96 and 100%, respectively, after 2 days; and both gave 100% control after 3 days. Confirm gave 66% control after 2 days, reaching 100% control after 4 days. Dipel was slower against third and fourth instars than against younger larvae, providing 6, 66, and 84% mortality after 1, 4, and 5 days.

Toxicity of Weathered Residues on Foliage

Feeding of midsized ETC on cherry leaves with 3-day-old weathered insecticide residues resulted in 100, 73, 39, and 20% mortality for Talstar, Spintor, Dipel, and Confirm, respectively, after 48 hours. Similarly, feeding on 7-day-old residues of those products gave 100, 59, 23, and 16% mortality, respectively, after 48 hours. Talstar clearly worked fastest and had the longest residual effectiveness of the insecticides we tested. For example, 3-day-old residues of Talstar gave 80% control after 1 day, and 100% within 2 days. Mortality for larvae fed similar-aged residues of Dipel (*Bacillus thuringiensis*) was 6, 39, 48, and 81% after 1, 2, 3, or 4 days, respectively. Larvae fed 7-day-old Talstar residues suffered 47 and 100% mortality within 1 or 2 days, respectively, whereas 7-day-old Dipel residues gave only 6, 23, 35, and 52% mortality after 1, 2, 3, or 4 days, respectively.

Test for Behavioral Avoidance of Residues

ETC showed no behavioral avoidance of residues of any of the insecticides. Numbers of pairings (counting only those in which the larva fed) wherein the treated leaf had more damage than the control were 5 of 9 for Talstar, 13 of 25 for Dipel, 14 of 24 for Spintor, and 11 of 20 for Confirm. The low number of total pairings for Talstar reflects the relatively high mortality in that treatment.

Micro-Injection of Cherry Trees with Dicrotophos (Bidrin)

Micro-injection with bidrin was very effective when the treatment was done early (16 April), when most (about 90%) of the ETC were second instars and the tents were small. All 50 nests harvested from control trees were viable and robust. Those nests contained a mean (± SE) of 304 ± 91 live larvae each (range 47 to 978). Of the 50 sampled nests from treated trees, 33 contained all dead larvae, and the rest contained just a few survivors. Collectively those 50 nests contained only 136 live larvae. The 16 April treatment therefore gave about 99% control.

The ETC population was developmentally more advanced when the original control trees were injected on 23 April. At that time, the mean proportions of second, third, and fourth instars present were 17, 63, and 20%, respectively. The 50 nests sampled from those trees on 27 April contained means of 190 ± 29 dead or moribund ETC versus 266 ± 37 active larvae, representing about 42% control. Larvae were mostly fourth and fifth instars by that time.

Test for Residual Control of Wandering Larvae in Pastures

Treating pasture grass with Sevin or Malathion gave almost no residual control. Numbers of live larvae recovered after confinement on freshly treated grass for 24 hours were 15.5 ± 0.9, 13.7 ± 1.7, and 12.8 ± 1.7 for control, Sevin, and Malathion, respectively. Corrected mortality, based on the proportion of dead versus live larvae, was <5% for each treatment. Totals of 50, 47, and 44 live larvae were recovered from those plots, respectively, after 72 hours.

Preliminary Test for Residual Toxicity of Bifenthrin to ETC on Tree Trunks

This method appeared ineffective. ETC crawling from the tops of the cherry trunk sections (where they had been placed) invariably dropped to the ground as, or before, they encountered the insecticide-treated bark and appeared unaffected. The assay may not have realistically simulated behavior of larvae that are behaviorally predisposed to crawl down tree trunks. However, the outcome is consistent with observations of ETC dropping from, as well as crawling down, trees in the field.

Conclusion

The prolonged emergence period of ETC complicates its management with short-residual insecticides. Soaps and oils, which have no residual, must be sprayed directly on target insects and therefore are ill suited to this problem. They do not penetrate the silken nets, and getting adequate coverage in tree canopies is problematic. *Bacillus thuringiensis* (Dipel) works well against small ETC but is slower and less effective against late instars. Because it has relatively short residual activity, three weekly applications, starting a few days after first egg hatch, likely would be needed for a high degree of control.

Talstar (bifenthrin) was the most effective, fastest, and longest-lasting foliar insecticide that we tested. It controlled both small and large larvae, so by waiting until most of the ETC population had emerged (two to three weeks after first egg hatch), farm managers likely could obtain excellent control with one application. SpinTor (spinosad) was nearly as effective, but label restrictions (see below) may limit its versatility for horse farms.

Both the Environmental Protection Agency's (EPA) 1992 Worker Protection Standard and label grazing restrictions limit the range of insecticides that can be applied to trees on horse farms. Dipel can be used on such sites, with no grazing restrictions. Of the several bifenthrin formulations, Talstar Nursery Flowable is labeled for agricultural use sites, including trees on horse farms (G. Meinke, personal communication). It carries no grazing restrictions (but it is not labeled for direct application to pastures). Grazing restrictions on spinosad labels (Conserve®, SpinTor®) limit its application to sites where drift onto pastures will not occur. Farm managers and applicators should read labels and consult regulatory officials to ensure compliance with label restrictions.

Micro-injection of trees with bidrin was highly effective against young ETC but less so against larger larvae. Bidrin is quite toxic to mammals, but micro-injection by a certified arborist allows it to be delivered systemically, with no drift and low hazard to livestock or farm workers. Studies planned for 2003 will evaluate less inherently toxic systemic insecticides (e.g., abamectin) and delineate the temporal window during which such applications are effective.

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Use of Recommended Insecticides Tebufenozide and Spinosad in the Vicinity of Broodmares

K. McDowell

IN THE EPIDEMIOLOGICAL SURVEY OF 133 KENTUCKY horse farms following the 2001 Mare Reproductive Loss Syndrome (MRLS), presence of black cherry trees and eastern tent caterpillars (ETC) were significant risk factors for mares that lost their pregnancies (1). Thus, by late summer of 2001, the University of Kentucky College of Agriculture recommended control of ETC for reducing or preventing MRLS in the future (2,3). Two of the insecticides, tebufenozide (Confirm®, Rohm & Haas, Philadelphia, PA) and spinosad (Conserve®, DowElanco, Indianapolis, IN), were relatively new products and as such may not be as familiar to horse farm owners and managers as some of the more traditional insecticides. Therefore, this project was initiated to address questions from local horsemen about the use of these newer insecticides in the vicinity of horses.

Tebufenozide and spinosad are biologically active insecticides and are recommended for control of ETC (3). The insecticidal activity of both of these products is expressed primarily through ingestion by caterpillar larvae. They are designed to be sprayed onto tree leaves on which the caterpillars feed. Thus, the question was raised regarding possible wind drift of the insecticides and hence accidental ingestion of small amounts of the products by grazing horses.

No reports were found in the literature where these insecticides had been used specifically in the vicinity of horses, and at the time this project was conducted (February of 2002), neither product was approved for use on pasture or with grazing animals. However, for the sole purpose of this research project, the insecticides were applied directly onto the hay fed to pregnant mares.

Materials and Methods

Sixteen pregnant mares, approximately 250 days gestation, were divided into two groups of eight mares each. Both groups were maintained in adjacent pastures and supplemented with hay fed on the ground. Tebufenozide and spinosad were mixed together with water in a 2-gallon sprayer, at rates suggested by the manufacturers. The insecticides were sprayed directly onto the hay fed to one group of eight mares. Water was sprayed onto the hay fed to the second group of eight mares. Hay was sprayed to the point that the hay was slightly dripping. It was treated on Monday, Wednesday, and Friday for one week (three treatments total).

All mares were palpated, their pregnancies examined by ultrasonography, and blood samples were taken before treatment started and 8 and 18 days after treatment started. Serum samples were analyzed for total estrogens, progesterone, and thyroid hormone (T4) (Bluegrass Embryo Transplants, Lexington, KY) and clinical chemistry (the University of Kentucky Livestock Disease Diagnosis Center [UKLDDC]). Data were analyzed by the Mixed Procedure of the Analysis of Variance (6) for main effects of treatment and day and the treatment by day interaction.

Results

The mares readily ate hay that was sprayed with either insecticides or water. No adverse effects were observed for the mares or for their pregnancies. All mares had normal healthy foals. All blood chemistry values were within

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acceptable ranges. Average glucose concentrations increased over time, but there were no differences between treatment groups (Table 1). Serum hormone concentrations are shown in Figure 1. Estrogen concentrations were higher on January 31 than on February 11 or February 21, but there were no differences between the treatment groups (Table 1).

Discussion

There is increasing evidence that MRLS is caused, in whole or in part, by ETC. Several methods are recommended for control of these insects, including spraying insecticides on trees on which the caterpillars are feeding (2,3). Many people are interested in safer, more environmentally friendly insecticides than those frequently used in the past. Two of the newer insecticides recommended by the University of Kentucky College of Agriculture for the control of ETC are tebufenozide and spinosad (2,3). Both are targeted toward specific classes of insects, not mammals, and their reported toxicities in mammals are extremely low (4,5).

Tebufenozide induces caterpillars to molt. Insects periodically shed their outer cuticle layers in a process called molt. Molting is initiated by an increase in levels of the steroid hormone 20-hydroxyecdysone (20E). 20E binds to its receptor, ecdysteroid receptor protein, and activates genes responsible for the molting process. Molting is terminated by a decline in 20E to basal levels. Tebufenozide is a metabolically stable ecdysteroid receptor agonist, and thus it induces the molting process. The caterpillars are unable to terminate this induced molt and die of dehydration or starvation. All molting insects utilize the 20E/receptor complex, but molt inhibitors such as tebufenozide do not function similarly with all insects. This selectivity may be due, at least in part, to different binding affinities of the compounds for the different insect systems. In addition, 20E and its receptor are not found in mammals; thus, 20E agonists cannot serve as specific ligand agonists in mammalian systems (4,7,8). 20E agonists have, however, also been found to affect membrane structure and function, presumably via significant lipophilic activity (9). Indeed, they have been reported to produce excitotoxicity by blocking K+ channels (10). Thus, there may be potential for neurotoxicity, independent of 20E/receptor activity.

Spinosad is a mixture of two naturally occurring metabolites of *Saccharopolyspora spinosa*, spinosyns A and D (11). Spinosad targets the insect nervous system, affecting gamma-amino butyric acid (GABA) receptor function, and causes involuntary muscle tremors, paralysis, and death. Differences in insect and mammalian nervous systems account for the reported safety of this insecticide for mammals (11,12).

Table 1. Mean, standard deviation of the mean (Stdev) and standard error of the mean (SEM) for each day tested.

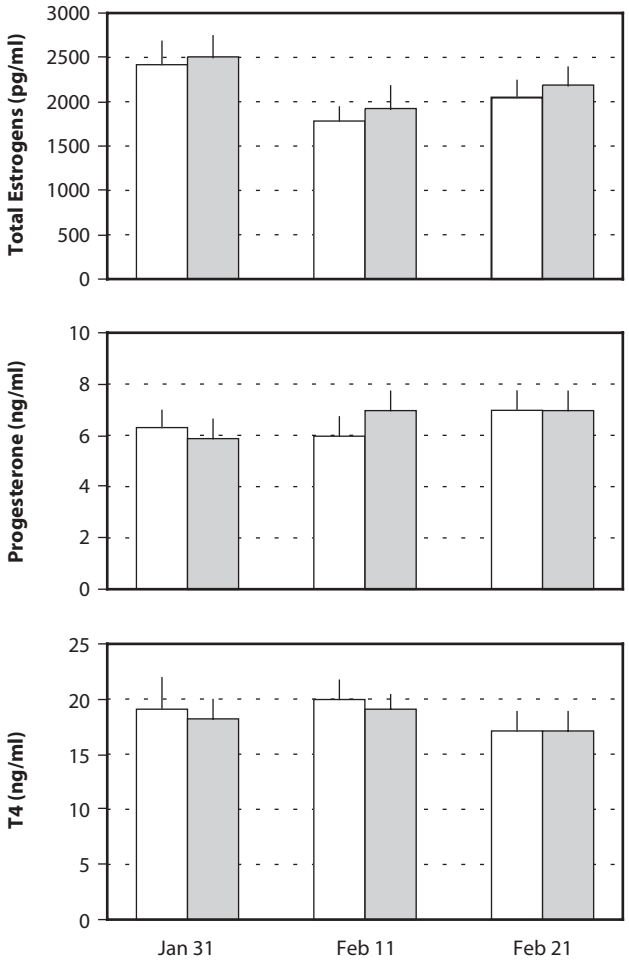
	Glucose			Estrogens		
	Mean (mg/dl)	Stdev	SEM	Mean (pg/ml)	Stdev	SEM
Jan 31	42	18	5	2464	687	172
Feb 11	66	20	5	1848	601	150
Feb 21	77	14	4	2116	550	138

Significance values for pairwise differences between dates.

	Glucose	Estrogens
Jan 31 vs. Feb 11	$p < 0.0305$	$p < 0.0002$
Jan 31 vs. Feb 21	$p < 0.0001$	$p < 0.0103$
Feb 11 vs. Feb 21	$p < 0.0550$	$p < 0.0542$

When these insecticides were sprayed onto hay that was fed to pregnant mares, no adverse effects were seen on the mares themselves, their *in utero* pregnancies, or on the foals that were born to these mares. All mares delivered live, healthy foals. Several of the foals have been weaned as of this writing and continue to be in good health.

Figure 1. Hormone concentrations in mares when hay was sprayed with insecticides (open bars) or water (shaded bars).



Both of these insecticides are designed to be sprayed onto the tree foliage where caterpillar larvae are feeding. They are ineffective if sprayed onto the caterpillar itself. They were only used on hay in this experiment as a direct test for effects on the horses, not as a means of caterpillar control. Package instructions should be followed when using any insecticide, and appropriate care should be taken to minimize human and animal exposure.

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Field Experiences with Caterpillar Control

S. E. Johnson

RECENT STUDIES HAVE ESTABLISHED THE RELATIONSHIP BEtween Mare Reproductive Loss Syndrome (MRLS) and the presence of the eastern tent caterpillar (ETC). Although the exact mechanism of the loss of foals and pregnancies attributed to MRLS still remains unknown, from a management standpoint, we now have sufficient information to begin to implement control measures for MRLS by controlling ETC concentrations on our farms.

Management Practices Initiated Prior to the Establishment of ETC as the Link to Mare Reproductive Loss Syndrome

There were several hypotheses as to the cause(s) of MRLS in 2001, and based on some of these theories, a great majority of the farms in Central Kentucky established contingency plans in 2002 in an effort to alleviate the high losses of early-term pregnancies and late-term foals that so many farms experienced in 2001. These contingency plans were based on what information was available to the industry after studying many of the factors of MRLS that were summarized in several surveys and field reports from 2001. Some of the areas into which researchers were looking were the ETC, mycotoxins, weather patterns, and how horses kept on pasture may be exposed to various viruses, bacteria, toxins, minerals, and other pathogens that could be the cause of MRLS.

Prior to the work that indicated the presence of ETC was responsible for MRLS, management practices were implemented on many horse farms in Central Kentucky early in the spring of 2002 in an effort to reduce losses to MRLS. These practices included:

- limiting the amount of time broodmares are exposed to pasture
- muzzling broodmares
- keeping broodmares in drylots
- spraying wild cherry trees with many different pesticides
- applying "sticky collars" around the trunks of trees
- attempting to pick out ETC egg masses from wild cherry tree branches
- "vaccinating" wild cherry trees
- burning ETC webs that were found in trees
- keeping pastures mowed as short as possible
- chain harrowing pastures
- cutting down and removing wild cherry trees
- moving broodmares out of Kentucky
- moving broodmares to different locations on the farm
- delaying breeding mares until after the middle of March
- adding binders to feed rations
- making different mineral supplements available to broodmares.

ETC Established as the Link to Mare Reproductive Loss Syndrome

As soon as it became apparent that ETC was the culprit, it became clear that many of the above practices had helped reduce the incidence of MRLS in 2002. Now breeders and managers could begin to have confidence in their efforts to limit exposure to ETC and not have to continue to employ such varied "shotgun" management techniques to control MRLS on their farms.

Currently we see farms taking strong measures to reduce exposure to ETC by removing the primary habitat of ETC, which is the wild cherry tree. These trees are being cut down, girdled, and killed with herbicides in an effort to control ETC.

ETC Control Measures

Additional study and work is needed to find other means of control for ETC. The chemical controls, while somewhat successful, still have limitations because of the vast expanse of wild cherry trees throughout the region, the difficulty in spraying many of the larger wild cherry trees, the exponential concentrations of ETC during some years, the migratory nature of the caterpillar, and environmental and toxic effects of some pesticides. Even though many farms implement control measures within their borders, if their neighboring farms do not have ETC control practices, then many caterpillars migrate across property lines and cause problems to the farms that are working hard to control ETC on their property.

Several farms reported a great deal of success "vaccinating" their wild cherry trees with systemic pesticides. This procedure involves injecting a measured amount of a pesticide into the trunk of a wild cherry tree, and when this pesticide is transpired throughout the tree, the early larvae feed on the leaves of the treated trees. When the young larvae emerge from their egg mass and feed on the treated leaves, they soon die.

An obvious area that will require further research and study is with insecticides. Although many farms had success with spraying insecticides on ETC, it was reported that the most success was when the young larvae were still in

Margaux Farm, Midway, Kentucky.

the trees and had just begun to feed on leaves that were treated with pyrethrins and/or carbaryl products. Although insecticides in the organophosphorus family may be highly effective, they have been shown to be toxic to mammals.

Conclusion

Now that we are aware of the connection between ETC and MRLS, and as helpful as it will be for researchers to learn the mechanism by which ETC causes MRLS, it is of paramount importance that future work be directed toward ways to control ETC. Principally, efforts should be made by the entomologists and the chemists to find safe and effective means with which to control ETC. By having stronger and more predictable techniques to be better able to control ETC, managers and horsemen will be better able to control the incidences of MRLS on their farms in the future.

Potential Uses for the Eastern Tent Caterpillar Sex Pheromone

K. F. Haynes

SEX PHEROMONES MEDIATE MATING BEHAVIOR FOR MOST moths. Typically the female moth releases a pheromone blend that stimulates the male moth to fly upwind. The eastern tent caterpillar (ETC) moth, *Malacosoma americanum*, fits this pattern. Females release a blend of (E,Z)-5,7-dodecadienal and (E,Z)-5,7-dodecadienol. Males are attracted to a blend of these compounds, but more work needs to be done to define the most effective pheromone blend. Pheromones have been used to help control insect pests in a number of different ways, and some of these could be used against ETC moths. Pheromone traps could be used to monitor flights of male moths. This could be useful in mapping the geographical distribution of ETC or following the long-term population cycles that characterize this species. Pheromone traps could also be used to define the end of a seasonal flight period, which could be useful if a decision is made to remove host trees after egg laying has occurred. With other species, pheromones have been used for more direct manipulations of insect populations. The trap-out tactic involves removal of enough males to limit reproduction. This approach is very labor intensive. Mating disruption involves wide dispersion of the sex pheromone resulting in inability of males to find females. Sometimes insecticides are combined with the pheromone to "attract and kill" males. Another possibility is to use pheromones as an aid in auto-dissemination of an ETC-specific virus. Males would be attracted to a pheromone source that contains a formulation of the virus. Males would subsequently contaminate females with the virus, who would contaminate the eggs. The success of tactics that use sex pheromones depends on the proportion of males affected, the area covered with the pheromone treatment, and the distance of migration of mated females. These methods have the potential to interrupt the annual life cycle of this insect, but action is required during the very short adult lifespan.

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Summary

T. D. Fitzgerald

WE HAVE QUITE A DIVERSE GROUP HERE IN TERMS OF THEIR interests, and how does one summarize these different ideas? I think two things come to mind. As Dr. Webb said earlier, the kind of research that Dr. Rieske-Kinney is doing would never have been funded several years ago. If you went to a granting agency and said, "I want to find out how far a caterpillar walks," I don't believe you would have gotten much money. This becomes extremely relevant, as this kind of basic information is very important. The other concerns control. It seems to me that there shouldn't be an easier insect to control than the ETC. What we need is a laser gun to target the eggs and blast them out of the trees. Another possibility is a small capsule that you shoot into the tent. It would open, and a volatile chemical would come out and saturate. Those caterpillars will die because they come back to the tent, they love that tent, and they won't leave it. Why cut down the tree? Well, it's like throwing the baby out with the dirty bathwater. It's overkill. Appropriate technology may be a possibility here, and there's a good opportunity to develop it.

State University of New York, Cortland, New York.

Session 8

Mare Reproductive Loss Syndrome Monitoring Programs for 2002

Chairperson: Dr. M. Scott Smith, Dean, College of Agriculture, University of Kentucky

Overview of the Mare Reproductive Loss Syndrome Monitoring Program for 2002

W. Long, J. C. Henning, B. Coleman, L. Lawrence, C. Peterson, and A. Reinowski

DURING THE SPRING OF 2001, THE KENTUCKY HORSE industry experienced a severe and sudden outbreak of early fetal loss (EFL) and late fetal loss (LFL), birth of weak foals, and cases of pericarditis and unilateral uveitis. Barren and maiden mares, early foaling mares that were bred in February and March, and late foaling mares bred in April and May of all breeds were affected. These conditions were collectively termed Mare Reproductive Loss Syndrome (MRLS), whose total economic loss to Kentucky exceeded $300 million.

The sudden and widespread occurrence of MRLS indicated that the causal agent was probably environmental or pasture related. In spite of intensive sampling, assays of several pasture parameters failed to show correlations with incidence of MRLS symptoms. Due to the sudden onset, it was impossible to sample pastures before fetal losses, making subsequent pasture assays difficult to interpret. A lack of baseline information about several pasture parameters also hindered interpretation. Farm visits during mid-May discovered a strong correlation of MRLS symptoms with the presence of black cherry trees (BCT), *Prunus serotina*, and exposure of mares to the eastern tent caterpillar (ETC), M*alacosoma americanum.* Other common pasture characteristics associated with MRLS included large amounts of white clover (*Trifolium repens* L.). Tall fescue (*Festuca arundinacea* Schreb.) was noted in several but not all MRLS fields.

Over the late summer and fall of 2001, several theories were advanced as to the potential causal agent for MRLS. These included cyanide content of ETC, cyanide content of white clover, ergopeptide alkaloids of tall fescue, mineral imbalances of pasture, nitrate content of pasture, and fungal mycotoxins associated with Fusarium and other fungi. In addition, late frost events during 2001 seemed to be coincidental with onset of MRLS. The incidence of poison hemlock (*Conium maculatum*) was also advanced as a possible cause for MRLS.

A monitoring plan was developed to follow these and other pasture and environmental parameters during 2002. This plan was developed with input from university scientists, private consultants to the equine industry, veterinarians, and farm managers. The goal of the monitoring program was to define "normal" levels of several pasture parameters thought to be related to MRLS and to monitor their impact on susceptible populations of horses (barren or maiden mares bred early or mares foaling after May 1).

Materials and Methods

In the fall of 2001, the University of Kentucky College of Agriculture outlined a monitoring system to follow pasture and weather parameters in Central Kentucky for 2002. From February 21 to June 30, 2002, data were collected from 13 Central Kentucky farms (12 horse farms and one hay production farm) on a biweekly basis to determine their potential relationship or correlation with MRLS. Monitored horse farms were selected to include both high-risk and low-risk Thoroughbred and Standardbred farms in Bourbon, Fayette, Jessamine, and Woodford counties. Risk level was determined by MRLS losses in 2001. Fields for each horse farm included barren or maiden mares and late foaling mares (April-May foaling). The monitoring program followed specific groups of mares over the duration of the sampling period and therefore would sample several pastures over the course of the season. Fields for the hay production farm included an alfalfa (*Medicago sativa*) and timothy (*Phleum pratense*) field. Data collected on mares included mare identity, breeding dates, reproductive status, clinical signs, and if any MRLS symptoms were experienced in 2001. A subset of horse farms collected blood and urine samples on a voluntary basis. These samples were stored for future analysis.

Additional fields from monitored farms were also sampled if MRLS symptoms occurred. Other farms (six) outside the monitoring program were monitored on a request basis after MRLS symptoms occurred. Field management history was recorded for all fields monitored and included fertilizer, lime, and herbicide application and rates, mowing frequency, and height. The presence or absence and management of BCT and ETC were also recorded.

Visits were made every two weeks for the majority of the season and weekly during late April and early May. At each visit, forage and soil samples were taken to characterize each pasture for loline and ergopeptides alkaloids (both in tall fescue alone and in the composite pasture), nitrate, cyanide (white clover only), fungal mycotoxins, soil yeasts and molds, forage minerals, and soil minerals. In pastures where BCT were present, samples were taken for pasture ergopeptides, fungal

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mycotoxins, and soil yeasts and molds in and out of proximity to BCT.

Forage samples were clipped manually and placed in paper bags except for white clover, which was placed in plastic bags. Samples for loline, ergopeptide, and forage mineral and nitrate analysis were placed daily in a forced air oven and dried at 50°C. White clover samples were kept refrigerated until analysis. Samples for fungal mycotoxin (forage tissue) and microbial population characterization (soil) were placed in paper bags and refrigerated until analysis.

Loline and ergopeptide analysis was done by gas chromatography (GC) and HPLC techniques, respectively. Fungal mycotoxins were measured initially by TLC and HPLC techniques and in the latter part of the sampling period by ELISA methods. Yeast and mold counts were determined by plate count methods.

Forage nitrate was analyzed with colorimetric techniques using cadmium reduction, and minerals were analyzed by atomic absorption methods. Cyanide levels in white clover were determined by standard EPA colorimetric procedures. Individual farms also collected rainfall as well as daily minimum and maximum temperatures from March through June.

Additional samples representing the composite pasture forage were taken from each pasture during each cycle and frozen to provide tissue for future analyses as needed based on future research or observations. For fields with BCT, two samples were taken: one from areas close to BCT and one from the remainder of the field.

Additional samples collected and stored or observations on a request basis included cyanide level of BCT leaves and flowers; ETC larvae, pupae, frass, and tents; and poisonous plants, including members of the Apiaceae family (*Conium maculatum*, poison hemlock). All samples were delivered to appropriate labs or frozen and stored on a daily basis.

The monitoring program included the formation of an oversight committee composed of University of Kentucky scientists and veterinarians, veterinarians in private practice and with the Kentucky Department of Agriculture, as well as horse farm managers. The role of the oversight committee was to oversee and advise the University of Kentucky as to any needed modifications in the monitoring plan and to provide feedback from affected and interested groups.

Results

From February to June, 75 fields were monitored as a part of the routine sampling program with additional fields added as required for follow-up of EFL or LFL. Routine soil samples were taken from each field at the start of the program. Loline and ergopeptide assays were conducted on 631 samples (260 from tall fescue alone and 371 representing total pasture species). Fungal mycotoxins and microbial counts were assayed on 402 and 400 samples, respectively. Nitrate-N and cyanide levels were measured on 246 and 244 samples, respectively.

Half (six) of the 12 horse farms on the routine monitoring program experienced MRLS losses (32 EFL and 9 LFL). These losses were associated with 13 different fields and occurred from April 25 to June 13. Of those fields with losses, only three did not have BCT either in or near them. All follow-up visits to additional farms with MRLS losses (from veterinary referrals) found potential for exposure to ETC.

Ergopeptide Alkaloids

Ergopeptide (ergovaline plus ergovalinine) results are reported for tall fescue alone (Figures 1 and 2) and for the composite pasture sample (Figures 3 and 4). Seasonal trends for total ergopeptides in tall fescue were low (< 0.500 ppm) prior to 11 April and peaked between 23 and 30 May (Figures 1 and 2). The trend was similar in fields

Figure 1. Seasonal levels of ergopeptide alkaloids in tall fescue alone from fields without BCT.

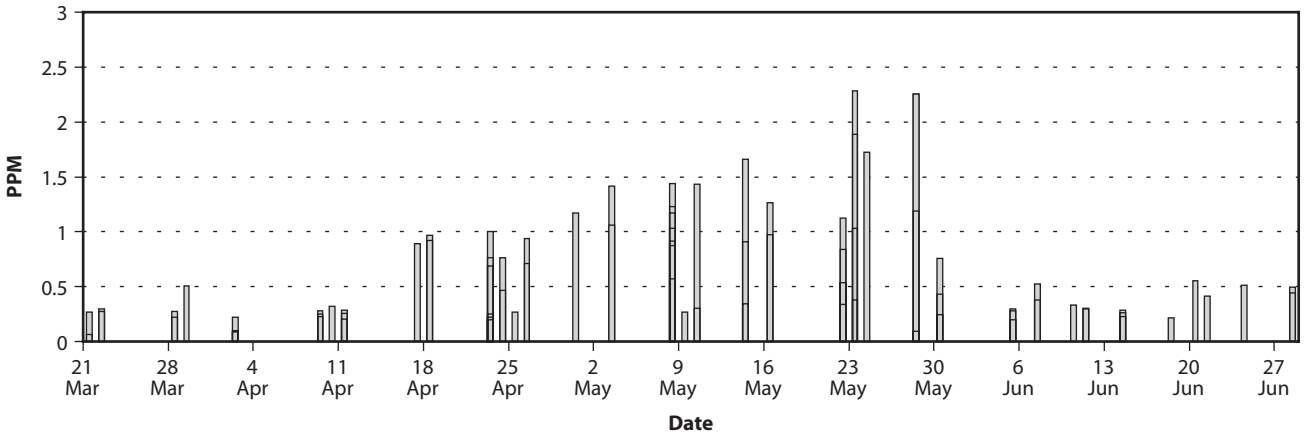


Figure 2. Seasonal levels of ergopeptide alkaloids in tall fescue alone in fields with BCT.

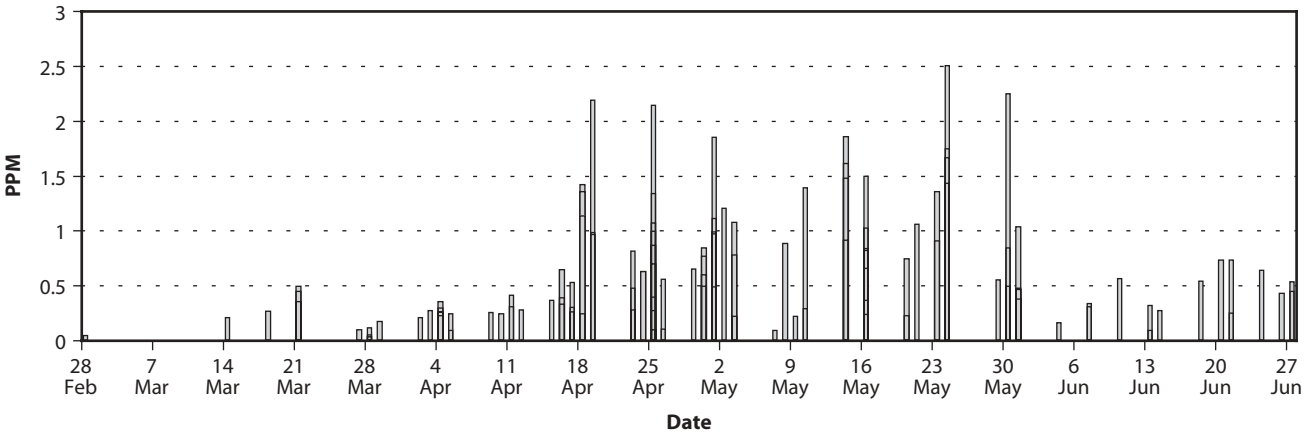


Figure 3. Seasonal concentrations of ergopeptide alkaloid concentration in total pasture forage in fields without BCT.

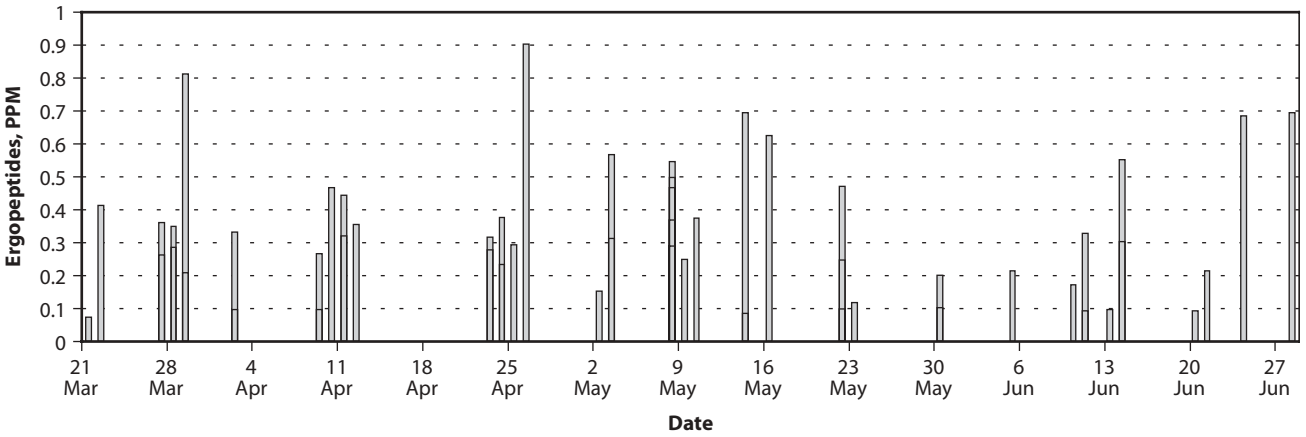
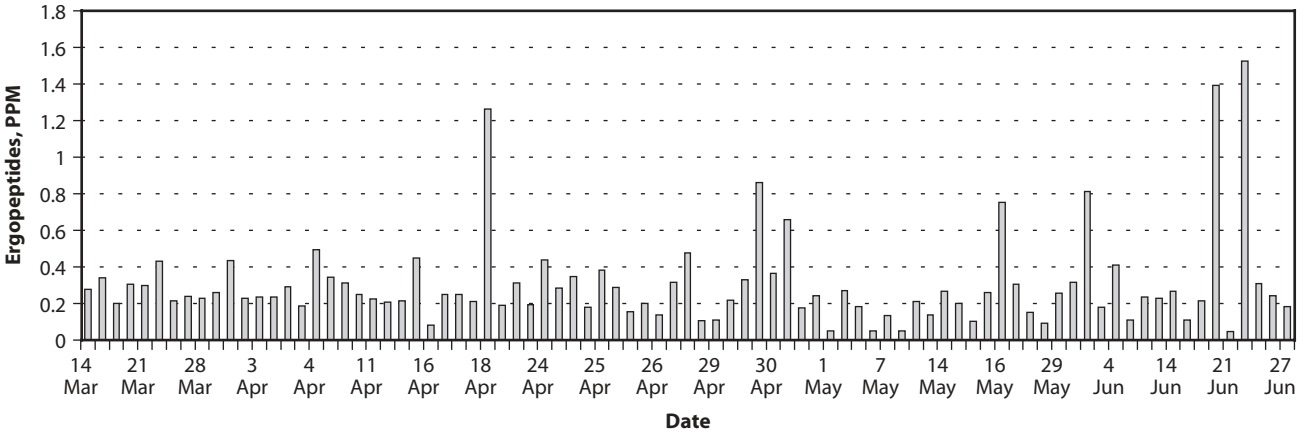


Figure 4. Seasonal concentrations of ergopeptide alkaloid concentration in total pasture forage in fields with BCT. Values are averages of samples in and out of proximity to BCT.



with and without BCT. In two instances in late June, the total ergot alkaloid levels were higher in the composite pasture samples than in the tall fescue alone (Figure 4 versus Figure 2). EFL and LFL did not increase with rising ergopeptide content in either tall fescue alone or the total pasture (Figures 5 and 6).

Fungal Mycotoxins

Only 63 of 402 samples had measurable dioxynivalenol (DON), T2, or zearalenone (ZEA) levels, and fields without BCT had positive mycotoxin results more frequently than those with (Tables 1 and 2). The majority of MRLS losses occurred on fields with no detectable mycotoxins (Table 3).

Soil Microbiology

Total yeasts and molds (Y+M) were variable and did not exhibit a seasonal trend with total populations between 10^5 and 10^6 colony-forming units per gram (CFU g^{-1}) (Figures 7 and 8). Counts tended to be greater in fields without BCT. Total Y+M counts did not impact MRLS incidence (Figure 9).

Nitrate, Forage Minerals, and Cyanide

Levels of nitrate-nitrogen (NO_3-N) were generally less than 1,000 ppm and 1,500 ppm for fields with and without BCT, respectively (Figures 10 and 11). Fields with BCT had more occurrences greater than 1,000 ppm than those without. While significant EFL losses did occur in some fields with high nitrate levels, nitrate had no consistent relationship with MRLS symptoms (Figure 12).

Forage samples from all pastures were analyzed for calcium (Ca) and potassium (K) content. In addition, routine soil tests were made for each field as it became part of the monitoring program. Limited numbers of samples were analyzed for sodium (Na), but these were uniformly low (approximately 40 ppm, data not shown). One theory of MRLS in 2001 was a mineral imbalance between K and Ca in forage, possibly exaggerated by late freeze events. Season-long values for potassium:calcium (K:Ca) ratio varied between

Figure 5. The effect of ergopeptide alkaloid content of tall fescue alone on EFL and LFL across all fields.

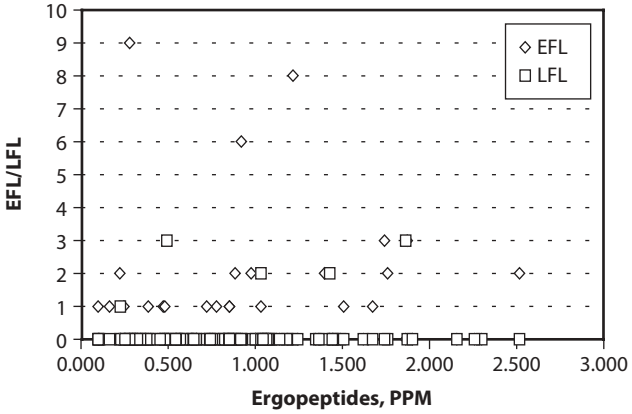


Figure 6. Effect of ergopeptide alkaloid concentration in total pasture forage on EFL and LFL across all fields.

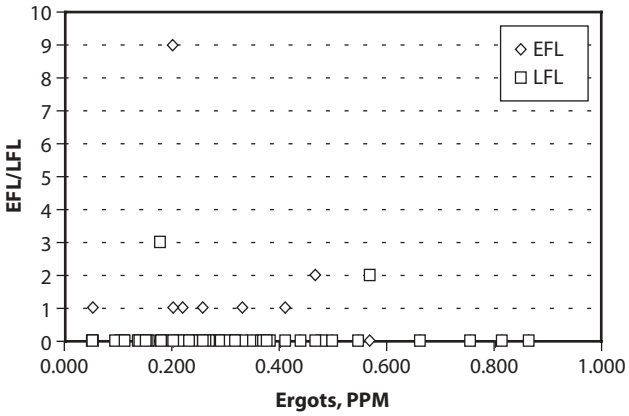


Figure 7. Seasonal populations of yeasts and molds in fields without BCT.

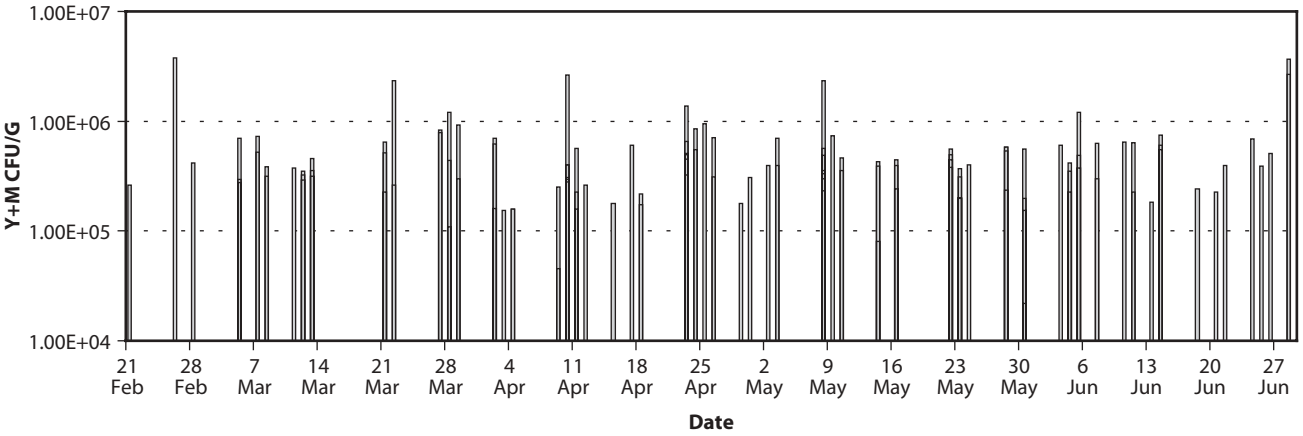


Table 1. Seasonal mean, minimum and maximum levels of mycotoxins present in fields with BCT (ppb). Values are averages of samples in and out of proximity to BCT.

	DON			T2			ZEA		
Week of	**mean**	**min**	**max**	**mean**	**min**	**max**	**mean**	**min**	**max**
18-Feb									
4-Mar									
18-Mar									
1-Apr									
15-Apr				131	100	173	469	356	574
29-Apr	733	700	1100	173	104	205	420	420	420
13-May	850	800	900	122	122	122			
27-May	833	700	1100	157	104	189			
10-Jun	600	600	600						
24-Jun	600	600	600	138	138	138			

Table 2. Seasonal mean, minimum and maximum levels of mycotoxins present in fields without BCT (ppb).

	DON			T2			ZEA		
Week of	**mean**	**min**	**max**	**mean**	**min**	**max**	**mean**	**min**	**max**
18-Feb									
4-Mar									
18-Mar									
1-Apr									
15-Apr				143	123	171	449	349	548
29-Apr				148	112	202			
13-May				126	103	149			
27-May	850	800	900	166	112	220			
10-Jun	700	700	700						
24-Jun				132	132	132			

Figure 8. Seasonal populations of yeasts and molds in fields with BCT. Values are averages of samples in and out of proximity to BCT.

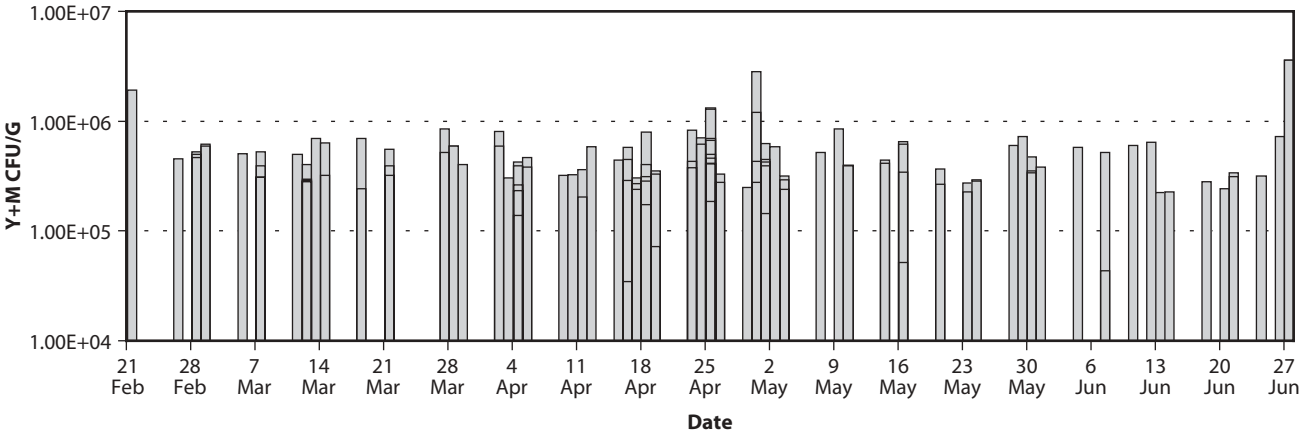


Figure 9. Effect of yeast and mold count on EFL and LFL across all fields.

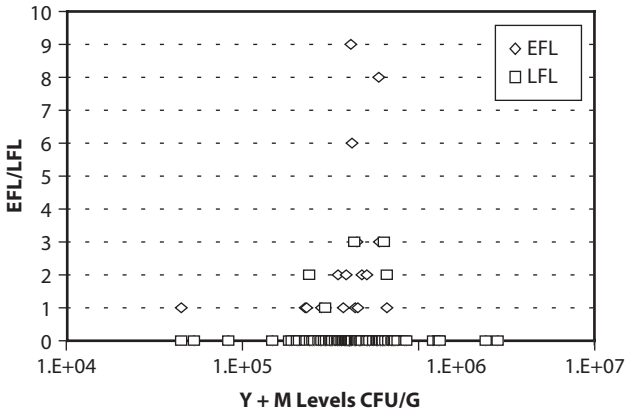


Figure 10. Seasonal nitrate nitrogen concentrations in total pasture forage in fields without BCT.

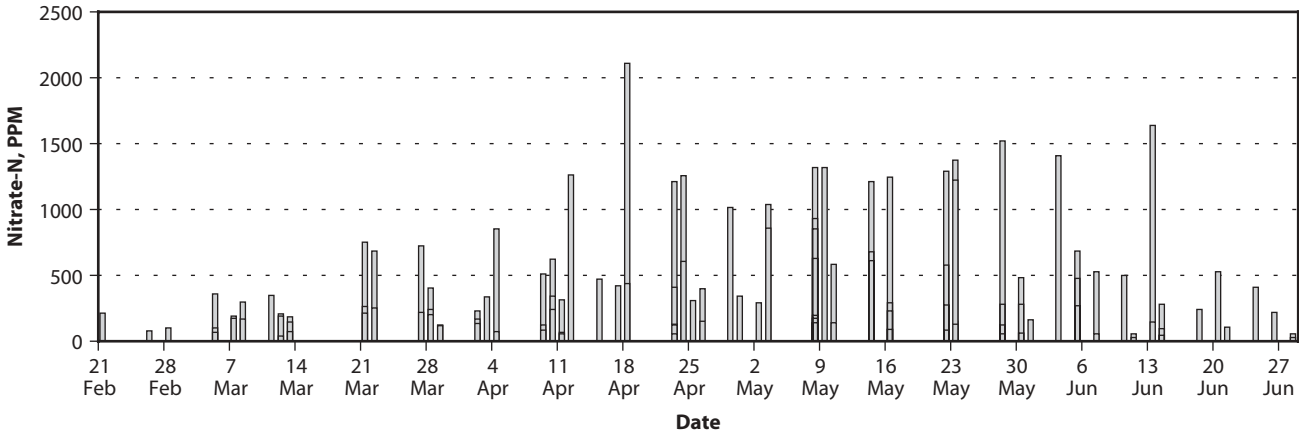


Figure 11. Seasonal nitrate nitrogen concentrations in total pasture forage in fields with BCT. Values are averages of samples in and out of proximity to BCT.

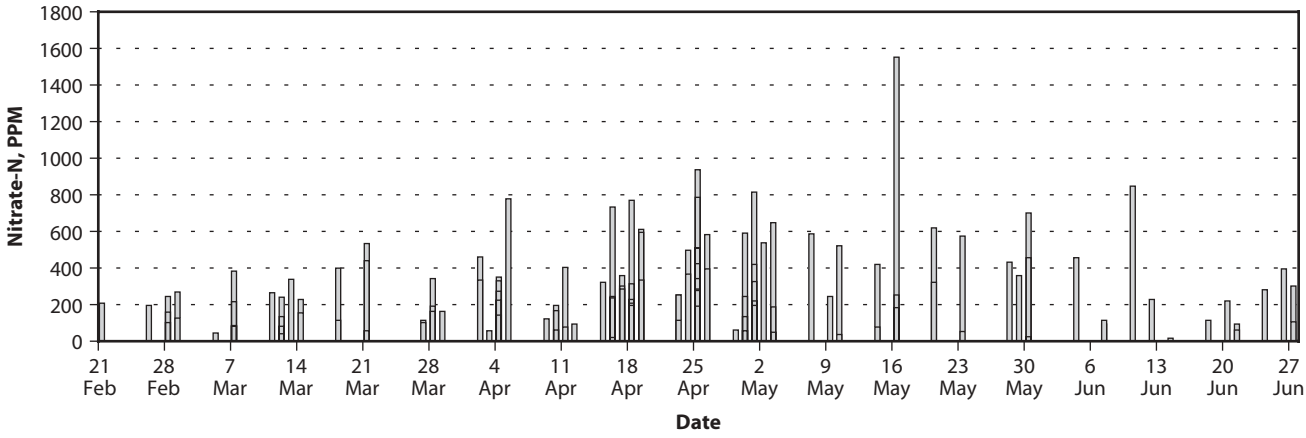


Figure 12. Effect of nitrate nitrogen on EFL and LFL across all fields.

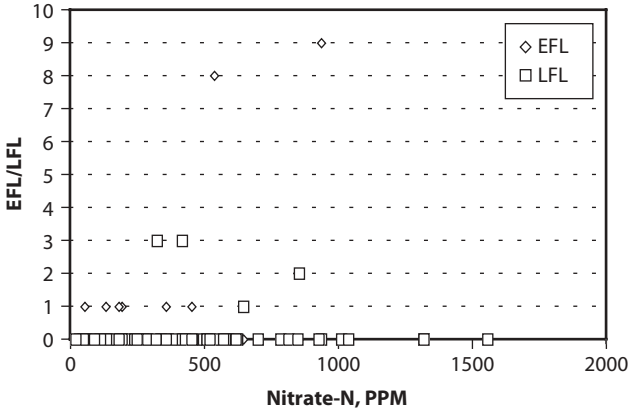


Figure 13. Seasonal K:Ca ratios for fields without BCT.

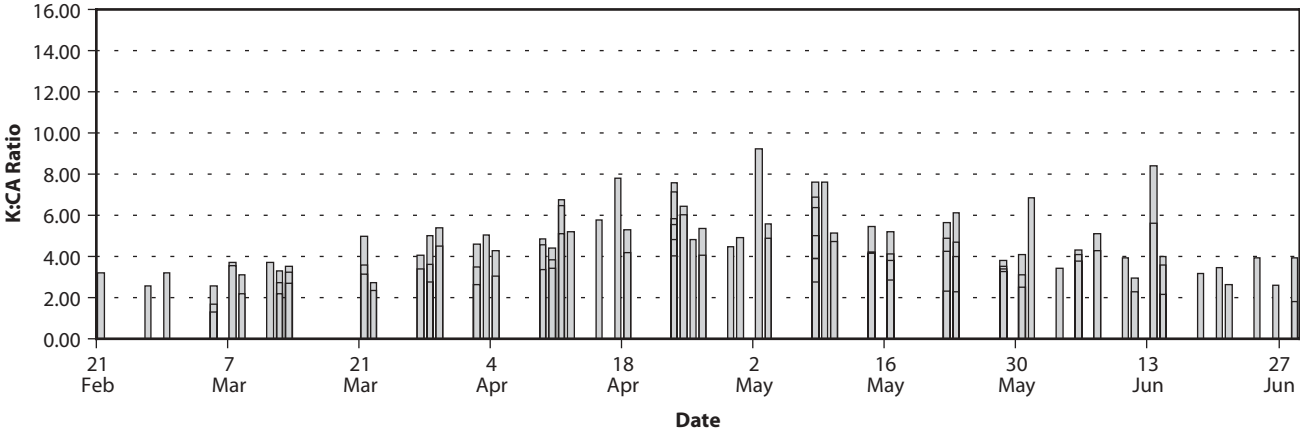


Figure 14. Seasonal K:Ca ratios for fields with BCT. Values are averages of samples taken in and out of proximity to BCT.

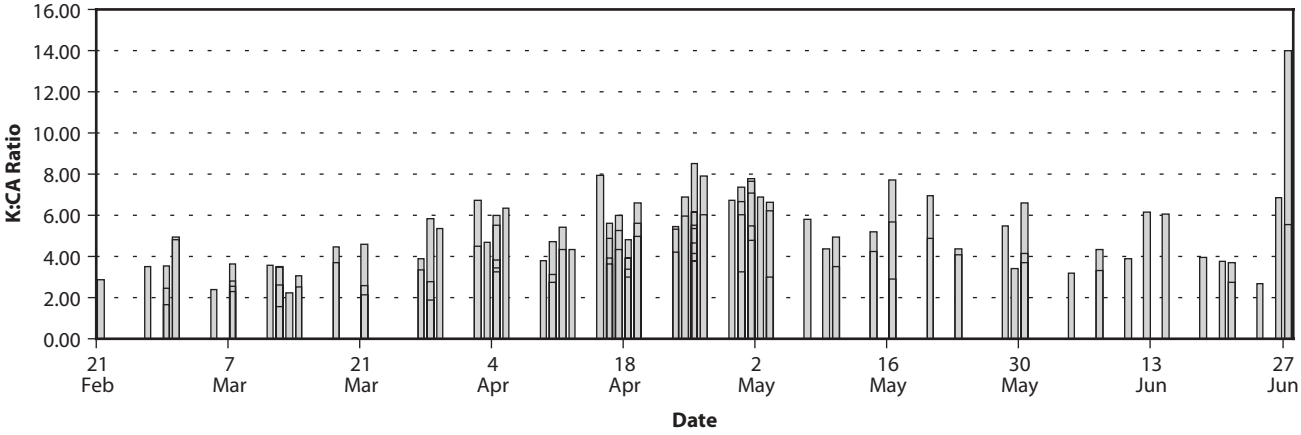


Figure 15. Effect of K:Ca ratio on EFL and LFL across all farms.

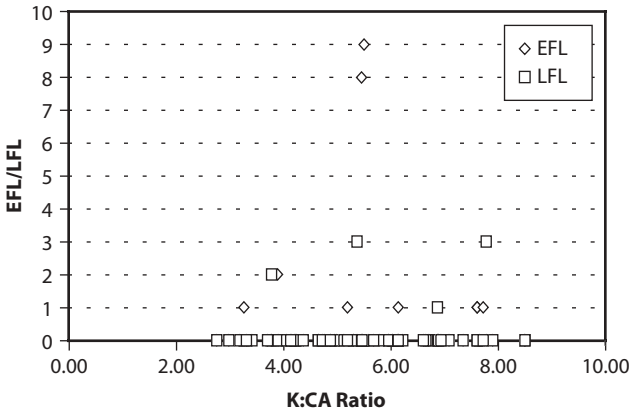


Table 3. Effect of mycotoxin level on total MRLS losses in 2002.

Mycotoxin Level	All Losses (EFL + LFL)
Below detectable limits	48
Positive DON, T2 or Zearalenone	13

Table 4. Average, minimum and maximum soil pH, Ca, K, P, Mg and Zn levels for 75 fields of the 2002 MRLS monitoring program.

	Soil pH	Buf pH	Ca, lb/A*	P, lb/A	K, lb/A	Mg, lb/A	Zn, lb/A
Avg	6.5	6.7	4861.8	309.5	665.5	407.0	7.3
Min	5.7	6.3	3092.0	58.0	252.0	197.0	2.5
Max	7.1	7.1	13076.0	740.0	1579.0	657.0	28.2

* lb/A = pounds per acre

1.31 and 14.0 and were not correlated to MRLS incidence (Figures 13, 14, and 15). Soil test levels for most fields were high to very high in Ca, K, and phosphorus (P), normal for magnesium (Mg) and zinc (Zn), and had pH values that would support good forage growth (Table 4).

Where detected, cyanide levels in white clover varied from 6.40 to 816 ppm hydrogen cyanide (HCN) and did not seem to differ in fields with or without BCT (Figures 16 and 17). Incidence of MRLS came from fields with low values for HCN (Figure 18).

Discussion

The alkaloids of tall fescue (lolines and ergopeptides) are produced in association with the presence of the endophytic fungus (*Neotyphodium coenophialum*) (1). Ingestion of tall fescue infected with the endophyte can result in foaling problems, specifically agalactia, dystocia, and prolonged gestation (2). While thresholds are unclear, levels of total ergopeptides greater than 500 to 600 ppm in the total diet are considered to induce fescue toxicosis in late gestation mares (Lowell Bush, personal communication). Several fields had tall fescue that exceeded 500 ppm ergopeptides from late April to late May (Figures 1 and 2). The assay for ergopeptide alkaloids in the composite pasture sample (taken from several points in the field without respect for species) should be more representative of the total diet of the grazing horse (Figures 3 and 4). Several composite samples exceeded 500 ppm across the season. However, no symptoms of fescue toxicosis were observed in mares grazing these pastures.

The relationship between ergopeptide alkaloids and MRLS losses is not clear, and high levels of alkaloids did not necessarily result in abortions. However, in three fields that did not have BCT but that did experience losses (5 EFL, 2 LFL), ergopeptide levels were very high (0.8 to 1.7 ppm, data not shown). Although the data are not conclusive, it appears that high ergopeptide levels may have played a role in fetal losses in 2002.

The mycotoxin theory of 2001 involved the presence of fungi in problematic pastures whose growth was stressed first by drought and then by frost leading to the produc-

Figure 16. Seasonal HCN concentrations in white clover from fields without BCT.

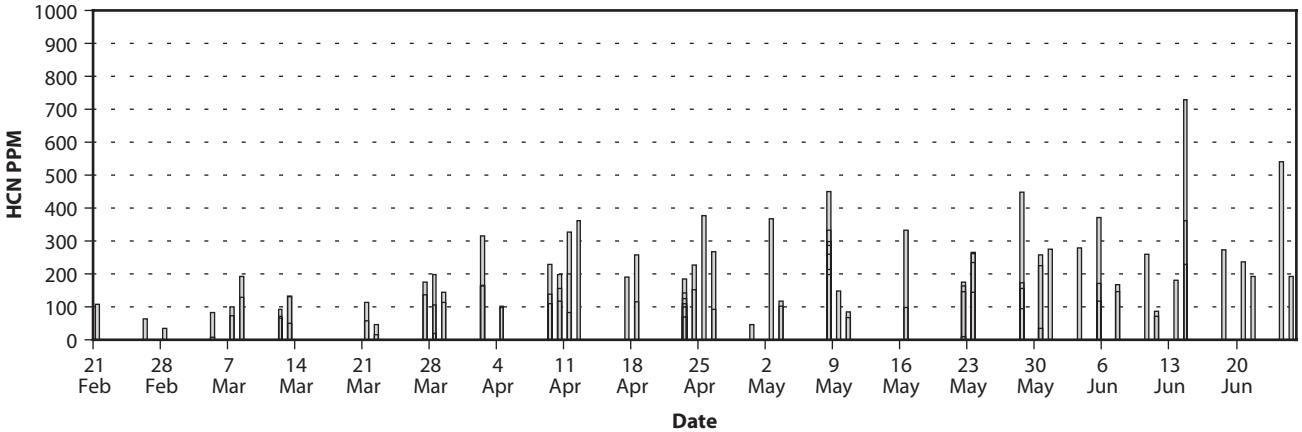


Figure 17. Seasonal HCN concentrations in white clover from fields with BCT. Values are averages of white clover samples taken in and out of proximity to BCT.

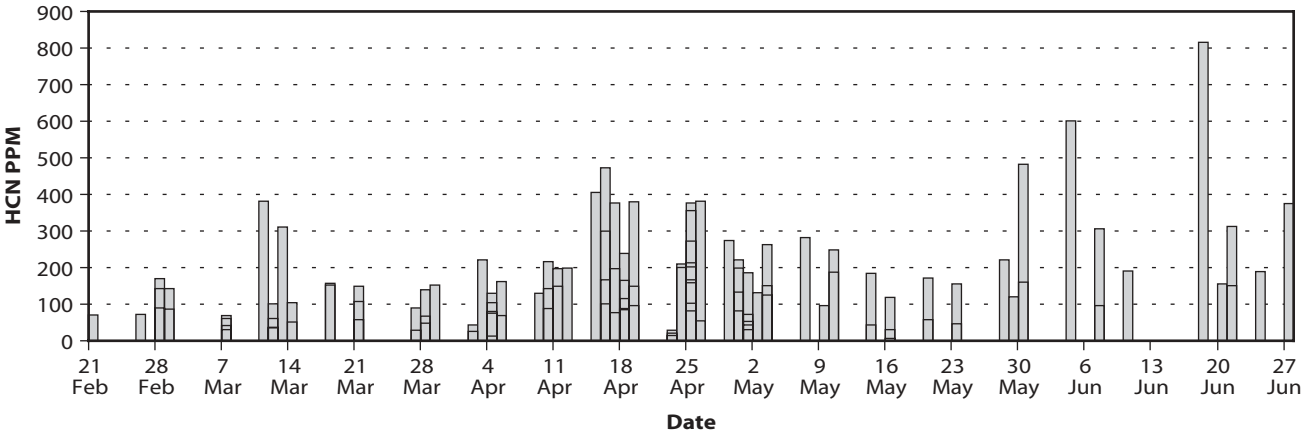
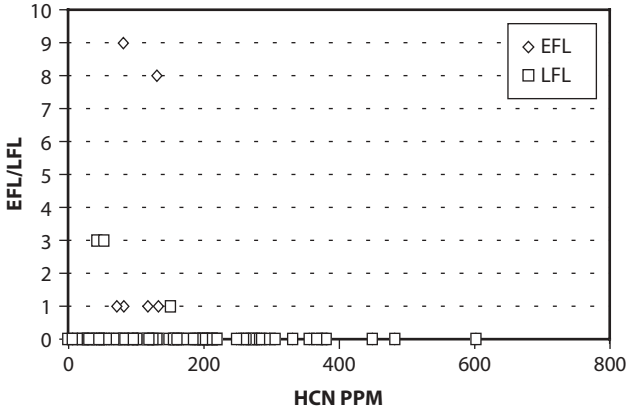


Figure 18. Effect of HCN concentration in white clover on EFL and LFL across all fields.



tion of mycotoxins that could cause MRLS. In 2001, rain events shortly after the Kentucky Derby hampered the ability to collect samples that would have been representative of the diet of mares experiencing MRLS (mycotoxins are water soluble). Therefore, it remained unclear if mycotoxins were produced in 2001 and what role they might have played in MRLS. Mycotoxins produced by *Fusarium* spp. were suspected but never found from the limited number of samples taken in 2001 during the MRLS episode. Samples taken in 2002 found that very few samples tested positive for mycotoxins, and these were not correlated with MRLS symptoms (Table 3).

Monitoring yeast and mold populations of the soil of horse pastures was thought to potentially predict problems should the conditions occur that favor mycotoxin production. Although fields with fetal losses were on the upper end of Y+M populations, there was not a clear relationship between fungal counts and MRLS occurrence (Figure 9). Therefore, it is doubtful that following microbial populations will provide a means of predicting the occurrence of MRLS.

Nitrate-nitrogen values of forage from monitored pastures increased from early March to some time in mid-April where the values seemed to plateau (Figures 10 and 11). In general, the values for nitrate content are higher than those reported during May of 2001 (Roger Allman, personal communication). The maximum amount of nitrate that horses can tolerate in their diet is not known, but Lewis reports that 4,090 to 4,770 ppm NO_3-N is known to be safe for pregnant or lactating mares (3). The values found in monitored pastures were well below this threshold (Figures 10 and 11). Finally, NO_3-N was not a predictor of EFL or LFL in 2002 (Figure 12).

The mineral hypothesis for MRLS advanced in 2001 was that a diet high in potassium, proteins, and carbohydrates and low in calcium and sodium chloride induced a mineral and electrolyte imbalance resulting in immune suppression and reproductive loss in mares. The suppression of the immune system would then lead to the overgrowth of the microorganisms found to be consistent with many cases of MRLS. Specifically, the K:Ca ratio was pointed to as the indicator of a problematic situation. In addition, it was hypothesized that the drought of 2001 and the late frosts would stress the plant leading to greater mobilization of potassium and hence greater K:Ca ratios. The K:Ca ratio ranged widely on monitor farms from 1.56 to 14 (Figures 13 and 14). These values were similar to those reported in May of 2001 (Roger Allman, personal communication). However, K:Ca ratio was not a good predictor of MRLS incidence (Figure 15).

The release of HCN from white clover (*Trifolium repens* L.) depends on the occurrence of cyanogenic glycosides (linamarin and/or lotaustralin) and the enzyme beta-glucosidase (4). Ordinarily these are compartmentalized, and

no HCN is released. However, when the tissues are damaged (such as by frost), the substrate(s) and enzyme mix, releasing HCN. The level of cyanogenic glycoside in white clover is genetically determined and varies by season. Many MRLS pastures in 2001 were characterized by higher than normal proportions of white clover. It was hypothesized that the late frost events that were coincidental with the onset of MRLS symptoms might have been related to release of HCN from white clover leaves damaged by the low temperatures. Cyanide release by white clover is increased by drought and by low temperatures (5). Mares were also observed to graze the clover areas preferentially in problematic pastures.

In 2002, cyanide levels in white clover ranged from 0.1 to 816 ppm across all fields (Figures 16 and 17). Crush and Caradus reported a range of 120 to 1,110 ppm HCN in 51 white clover cultivars grown under greenhouse conditions (4). In general, the cultivars of white clover used in the United States are low in cyanide potential, but some 2002 fields had HCN levels in white clover approaching those found in New Zealand, where cyanogenic potential is desired for improved agronomic performance and pest resistance (4,6). However, the EFL/LFL losses experienced by monitoring farms in 2002 were on fields with relatively low HCN levels in the clover (Figure 18).

General Farm Observations

Routine observations were made on monitored farms or on referral farms as to what and where the mares were grazing. Any poisonous plant found was brought to the attention of farm personnel. No poisonous plants were observed being eaten or bitten. From early March until the end of June, white clover was the preferred forage for all mares. It was also noted that mares would not graze white clover if it was mixed with tall fescue. Additionally, white clover mixed with timothy, orchardgrass, or bluegrass was selected over tall fescue or tall fescue-white clover mix.

Grazing generally was observed where the white clover population was the greatest, which, in most cases, was away from fences and tree lines. During the period of ETC migration, mares were observed, in some cases, several hundred feet away from BCT while grazing. However, ETC were also observed a similar distance from their host. ETC pupae were found in great numbers on fences between the board and the post. They were also noted in orchardgrass crowns well within the grazing height of mares and cutting height of hay production equipment. This condition may explain EFL losses that occur well after the migration was completed.

Ten of the 12 monitored horse farms had aggressive management approaches for controlling/eradicating ETC. They included complete removal of BCT, removing egg masses, spraying or injecting insecticides (organic and inorganic), and burning ETC tents. Other approaches included dry lots for mares, muzzling of mares, or completely moving the mares away from BCT. Losses occurred on six of the 12 horses farms regardless of management approach. Four farms experienced losses in spite of an aggressive management plan partly because of BCT outside of their control and greater than anticipated ETC movement.

Conclusion

The excellent cooperation among farm managers, veterinarians, private consultants, and laboratories and university personnel enabled unprecedented data collection on the environmental and forage characteristics of the pasture of horse farms in Central Kentucky in 2002. Baselines were established for several parameters in a coordinated way to allow comparison for future years and for future problems. One of the major problems in dealing with the MRLS outbreak in 2001 was the inability to define what "normal" would be for a range of pasture parameters, including alkaloids in tall fescue, fungal mycotoxins, soil microbial populations, cyanide, and nitrate. These and other parameters considered as possible causal agents of MRLS were measured on 12 Central Kentucky horse farms and one hay production farm. No measured parameter was observed to have a clear relationship to MRLS in 2002. Farms and fields with losses compared to those with no losses support ETC involvement in MRLS. ETC presence was therefore a good predictor of MRLS potential. However, there was some indication of problems related to LFL from very high levels of ergopeptides in tall fescue. Finally, the data collected during 2002 indicate that the number of pasture parameters that should be followed in the future can be reduced. Still needed, however, is knowledge of the toxic principle and how it is transferred to the grazing mare. Ideally, this compound or organism could be characterized so that horse farm managers are able to make sound decisions as to the safety of pastures for pregnant mares.

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Summary

J. Henning

IT IS VERY EASY TO SUMMARIZE A SESSION WITH ONLY ONE paper, so I will just let Wayne Long's comments stand. I would like to recognize the oversight committee for their helpful advice and consultation over the past year. I would especially like to recognize the invaluable efforts of Dr. Brown and Dr. Riddle, who helped us to craft the monitoring plan and to change it in midstream.

In addition, farm managers were with us: Fred Seitz, Dan Rosenberg, Art Zubrod, as well as Rusty Ford from the Department of Agriculture. Dr. Powell was ever present with words of wisdom and was a calming force in some tense meetings.

Let me just add that I was very pleased we were able to finally get some samples ahead of an event. I was worried this year that if we did in fact control all the caterpillars that we might not have a chance to correlate field experience against the MRLS problem, not that I wanted to see one, necessarily. But we do have prior forage, blood, and urine samples, as well as samples taken from within the time when mares were losing foals, so we can go back to try to construct what happened. This situation is a great improvement over 2001 when we took samples after the fact and tried to guess what the horses did or what they were doing when they lost the foal.

We were also able to respond, through our veterinarians, to a great number of farms that were experiencing losses for reasons they could not figure out. When we did the follow-up visits, of course we were looking for the standard items. We would take samples, and some of those are included in the measures Wayne Long presented. But we would have to agree with so many of the thoughts expressed that in some cases we've got losses not proportional to numbers of caterpillars. The three fields that had less obvious cherry trees had exceptionally high ergovaline and ergovalinine numbers. That's just one of the things that always bothers you when you put a theory together and try to find out if the data fit. There are times when you've got small trees or long distances from trees to horses on farms that had problems. There are also losses on farms that were adamant that thought they were doing a good job. So we've got some apparent outliers that, when we finally understand the vectors and mechanism, will make sense.

Finally, let's talk about 2003. I think any good monitoring program ought to do three things. First, it ought to predict a problem. In 2002, it was clear because of lag of analysis time and our "shotgun" approach that we probably weren't going to predict but respond. I think with the help of these two days, we've got a lot more ideas about the parameters we can monitor. Second, it ought to provide data for any theories as to the exact cause, and I think the 2002 samples should help us. Third, it ought to help farm managers answer the question "Is my pasture safe?" There's still a big challenge out there as far as answering that question, and I'm glad for some leads. Dr. T. Fitzgerald mentioned the Serratia. I'm glad we've got Dr. Newman on the team, who's a wizard at doing the super science stuff, as is Dr. Bush. Any monitoring program ought to predict, correlate, and then, after the fact, help people know when they can go back on pastures.

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Session 9

Workshop Summary

Chairperson: Dr. Keith Betteridge, University of Guelph, Ontario, Canada

Summary of Workshop Proceedings

K. Betteridge

OUR BRIEF FOR THIS CLOSING SESSION HAS BEEN VERY clearly set for us by Drs. Powell and Tobin. With a view to defining "where we go from here," we have been asked to center our discussion on three general areas:

- the approaches that seem most promising for identifying potential causative agents of Mare Reproductive Loss Syndrome (MRLS);
- suggestions for a useful laboratory model of the syndrome;
- cost-effective preventative measures for MRLS and for communicating these to all those affected by the condition.

I say "our" brief because you are going to do the work; I'm just the chair!

Because my job is not to summarize the meeting, I'm going to ask the summarizers of the various sessions to come forward and be available to answer questions as they crop up. However, before throwing the discussion open to the summarizers and to you on the floor, I must say that it would be churlish indeed not to take this opportunity to make some very appreciative comments about the meeting in general before getting down to the specifics of our mandate.

All of us have been very conscious of the fact that, as was said yesterday at Keeneland, there is nothing good about MRLS. Those of us coming from outside feel very sympathetic and very sad about those dreadful pictures we've been seeing of the problems you have been suffering over the past two years. I repeat, there is nothing good about MRLS; and yet, paradoxically, from the scientific point of view, this has been a most interesting workshop. As I've heard so many people say as we have been eating and talking outside, "If it weren't for its devastating effects, this disease would be great fun to investigate." But fun, it is not; important, it most certainly is.

Dean Smith set the scene for us in saying how people in Kentucky have been exemplary in three aspects of investigating MRLS: the investigation has been multi-disciplinary, it has been collaborative, and there has been great communication, both among the investigators and to the affected public.

Dr. Webb said how skeptical he was about this workshop—"How could it serve any useful purpose?"—and yet he has changed his mind during the meeting, and I heard him say that he sees it as a "positive move to integration." His words, I think, are evidence of the success of the initiative of Drs. Powell and Tobin and their colleagues in calling us all together.

As an outside observer, I've been enormously impressed by the efforts that have already gone into identifying potential causative agents. There has been what seems to me a most remarkable collaboration between the practitioners in the field and investigators in the laboratory. Needless to say, none of the investigations that we have heard about could have been done, nor could the whole picture have been characterized, but for the remarkable input of all the practitioners in the affected areas of Kentucky, and I think they deserve special praise for that.

There has also been commendable collaboration between various departments of the University of Kentucky and between the University and government in the investigation of MRLS, as was brought out very clearly at today's lunch.

Are eastern tent caterpillars (ETC) involved in MRLS? I would say that the consensus that is emerging from this meeting is that they are involved, although this is not universally accepted, and I think it is something that still needs to be debated. There have been interventions from the floor about infestations apparently in the year 2000, though others contend that they were nothing like as extensive or intense as in 2001. So, I don't think that the role for ETC is absolutely cut and dried. Remember, too, that Dr. Kronfeld's statistical summaries leave some doubt as to the strength of some of the experimental evidence for caterpillar involvement. In this connection, of course, we need to recognize the enormous difficulty of getting statistical significance out of the kind of numbers that we can work with—a key problem that we have identified and must cope with in the future.

If ETC are involved, then we have to know a lot more about them. Dr. Sebastian referred to ETC as "God's little creatures," quoting a Web site description of them. I went to William Shakespeare, *Richard II*, Act II:

> "… Bushy, Bagot and their complices,
> The caterpillars of the commonwealth,
> Which I have sworn to weed and pluck away."

(I thought that this was especially appropriate when I came past the Commonwealth Stadium on the way here).

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So, Bushy, Bagot, and all their friends certainly need to be better understood. If they are involved, the question is "How?" That question has been the focus of discussions—and I think very, very useful discussions—throughout the workshop. We heard about placentitis and funisitis; are they caused by a bacteremia from commensals, and, if so, is that bacteremia being initiated through the cervix or in some other way—through irritation of mucous membranes, perhaps? In fact, the portal of entry seems to be a key question that needs to be answered. Dr. Benirschke told us that in humans the portal of entry for any placentitis is via the cervix. In that context, I was intrigued to hear that the amniotic fluid seems to become infected and that infection doesn't seem to spread away from the amnion. Perhaps we need to remember that in all the talk of the placenta that we have heard over the past two days, we have really been talking about the fetal membranes. There are two sides to a placenta—the maternal and the fetal sides. I don't want to be pedantic, but I think that the fact that we don't really know anything (or very little) about what's happening on the maternal side of the placenta needs to be borne in mind, a point to which I will return in considering potential models of MRLS.

Again, in relation to fetal fluids, I think it's perhaps salutary to remember that the accumulation of fetal fluids is a very dynamic process, at least in pigs (1). Do analogous peaks in the accumulation of these fluids occur in mares? If so, might they indicate that there are portals opening between the conceptus and the mother, and might any opening of such portals constitute a route of entry for bacteria? The possibility that fluid movement might be important in MRLS is supported by the fact that the associated pericarditis also involves an accumulation of fluid over serous epithelia. Perhaps getting back to the basic physiology of how and when equine fetal fluids accumulate, expanding the studies of Arthur (2), could be useful, at least from an epidemiological point of view.

Much as been made of the fact that there have been no losses experienced in mares pregnant less than 35 days. I find this very interesting indeed, because I'm most interested in the conceptus up to 35 days. I would like to point out that it would be wrong to go away with the impression that there is no placental exchange before 35 days; there is, of course, but the nature of that exchange is very different, involving the bilaminar omphalopleure probably up until, and certainly after, the capsule disappears at about day 21. The rate of expansion of the conceptus should remind us all that there is exchange between the mother and conceptus right from the beginning of pregnancy, so don't let's forget that in thinking about the etiology of MRLS.

Certainly, there has been much thought about early detachment of the placenta being involved in MRLS. Dr. LeBlanc, for one, is a very strong advocate of this as being one of the ways in which the whole syndrome comes about. In relation to that, I wonder whether there is a place for some more detailed ultrasonography. I'm thinking here of Doppler ultrasonography that is being used to such good effect by Dr. Bollwein in Germany to measure blood flow to the uterus. It strikes me that if we do have a reproducible model—and it would have to be a reproducible model in the horse and not a laboratory animal—that perhaps getting at the changes in blood flow to the uterus could be very important. I think that the only sign of fetal distress that I heard about during the presentations (though I stand to be corrected) was an elevated heart rate in some of the experimental inductions. Zeroing in on the changes that precede abortion in this syndrome should be worthwhile, and if we could do so noninvasively with ultrasound, that could be very rewarding.

Some of the questions that have cropped up during the past two days should be answerable with relatively modest further effort. The weather data that are being amassed, for example, sound very promising for resolving the climatic background to MRLS and, in my view, should continue to be collected and analyzed. Like many others, I'm sure, I am intrigued by the unilateral uveitis found in MRLS. I must say I wonder why nobody seems to have simply passed caterpillars across some experimental eyes to see whether irritation is induced and leads to those changes. I thought it remarkable that there was no such eye effect, nor any pericarditis, in any of the experimental models that I heard about. Dr. Kronfeld's doubts about whether horses eat caterpillars should surely be relatively easy to confirm or refute. Dr. Kronfeld also underlined the need for a more generalized mycotoxin test; a nonspecific test would be very useful as a screening method to apply before looking for specific mycotoxins. Another relatively simple approach to investigating MRLS might be to look at the allergenic effects of caterpillars.

The call for a laboratory animal model system in which to study MRLS is urgent because of the cost of working with mares and the logistics of assembling enough mares to arrive at useful conclusions. While I agree entirely that we need a laboratory animal model, I would urge you not to get too diverted from the horse as the model of most direct concern because we need to remember the perennial problem of species specificity. You can prove all you like about such-and-such an agent doing such-and-such a thing in such-and-such an animal, but this will not necessarily be true in another species. Dr. Schlafer made the very important point that we are going to need to get at the maternal side of the placenta to go forward. Thus, we are going to need to sacrifice some affected animals; those affected animals, I would suggest, are going to have to come from some kind of reproducible model system. Another

exclusive opportunity that would be provided by a model of MRLS in horses directly would be that of facilitating the frequent blood sampling that is necessary to work out whether or not a bacteremia is associated with the syndrome. That question came up time and time again. If we had a treatment that we knew was going to be causing abortion in mares, there should be no limit to the effort that can go into monitoring them, if the money and the incentive are there. Thus, in my opinion, the work being done directly with horses should and must continue.

As to a laboratory animal model, immune-deficient mice would seem to have great potential (3). I can say from personal experience that growing trophoblast and endometrium together in such mice works beautifully. And so the right target tissue (e.g., placental tissue at a particular stage?) could be used and subjected to various treatments in these mice, making it a lot easier to achieve the kinds of statistics that we really need for MRLS investigation. Choosing the right tissue, of course, remains the $64,000 question.

There are, needless to say, some much more difficult and more long-term questions that I think we have to address. If Bushy and Bagot are involved, they have to be fractionated, and we have to find out which parts of them are involved. There has been much discussion about that. Drs. Webb and McDowell presented a very nice list of the steps that need to be taken to decide whether a putative agent is biological, environmental, or toxicological, and this approach has to be pursued.

The portal of putative entry is second to none in deciding whether we're talking about an agent that is hematogenous or not. Furthermore, it has been pointed out to me during the workshop that the possibility of a pulmonary route of entry being important received scant attention during our discussions.

In discussing control measures, Steve Johnson of Margaux Farm said that entomologists are now his "new best friends." I don't suppose that was so before this whole problem came up, and I take this as yet another example of the collaboration engendered by the investigation of MRLS.

I was struck by the significance of Dr. Volkmann asking Dr. Rieske-Kinney in which direction her ETC were migrating, experimentally. Coming from Cornell, he was obviously disturbed to think that they were going in a northerly direction; coming from Canada, I know how he feels!

I took Dr. Fitzgerald's mention of laser guns very seriously indeed. That struck me as an excellent idea, and I don't think it should be discarded because I think that the ecological consequences of wiping out cherry trees in this beautiful state would be catastrophic.

And finally, in this field of control measures, Wayne Long in the preceding session put his finger on the real nub of the question as far as the public is concerned: the paramount need to be able to identify safe pastures.

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List of Abbreviations

20E ..... hydroxyecdysone
ACTH ..... adrenocorticotropic hormone
AI/ha ..... active ingredient/hectare
ATP ..... adenosine triphosphate
AWC ..... Agricultural Weather Center
BCT ..... black cherry tree, *prunus serotina*. The common name for this species includes black cherry, wild black cherry, and wild cherry.
BID ..... twice a day
bpm ..... heart beats per minute
CFU ..... colony forming unit
CNS ..... central nervous system
CO_2 ..... carbon dioxide
CRT ..... corticotrophin-releasing hormone
DAS ..... diacetoxyscirpenol
DDT ..... dichloro-diphenyl-trichloro-ethane
DES ..... diethylstilbesterol
DMSO ..... dimethyl sulfoxide
DON ..... dioxynivalenol
eCG ..... equine chorionic gonadotrophin
EFL ..... early fetal loss
EFP ..... equine fibrinous pericarditis
EHV ..... equine herpesvirus
ELISA ..... enzyme linked immunosorbent assay
EPA ..... Environmental Protection Agency
ETC ..... eastern tent caterpillars (*Malacosoma americanum)*
EVA ..... equine viral arteritis
FAT ..... fluorescent antibody test
FSH ..... follicle-stimulating hormone
GABA ..... gamma-amino butyric acid
GnRH ..... gonadotropin releasing hormone
GC ..... gas chromatography
HCN ..... hydrogen cyanide
HCN-p ..... hydrogen cyanide potential
HIE ..... hypoxemic-ischemic (HIE)
HPLC ..... high performance liquid chromatography
H_2SO_4 ..... sulphuric acid
HR ..... heart rates
IgG ..... immunoglobulin G
IP ..... intraperitoneal
IU ..... international units
IV ..... intravenous
KCN ..... potassium cyanide
lb/A ..... pounds per acre
LD50 ..... lethal dose 50
LFL ..... late fetal loss
MRLS ..... Mare Reproductive Loss Syndrome
MRLS DDs ..... MRLS degree days
NaCN ..... sodium cyanide
NaOH ..... sodium hydroxide
NCDC ..... National Climate Data Center
NO_3-N ..... nitrate-nitrogen
NSAIDs ..... nonsteroidal anti-inflammatory drugs
NVSL ..... National Veterinary Services Laboratories
PCR ..... polymerase chain reaction
ppb ..... parts per billion
ppm ..... parts per million
PVC ..... polyvinylchloride
SID ..... once a day
SIRS ..... systemic inflammatory response syndrome
SPSE ..... septic penetrating setal emboli
T2 ..... trichothecene mycotoxin
TH-1 ..... thymus helper-1
TH-2 ..... thymus helper-2
TLC ..... thin layer chromatography
TTW ..... transtracheal wash
UKLDDC ..... University of Kentucky Livestock Disease Diagnostic Center
VDL ..... veterinary diagnostic laboratory
WBC ..... white blood count
ZEA ..... zearalenone

Bibliographies and Suggested Readings

Eastern Tent Caterpillar (*Malacosoma americanum*) Literature Having Potential Relevance to Managing Mare Reproductive Loss Syndrome

T. M. Leeson and D. A. Potter

Abstract

Recent research implicating exposure to eastern tent caterpillar (ETC), *Malacosoma americanum* (F.), as a risk factor in Mare Reproductive Loss Syndrome (MRLS) has sparked interest in the caterpillar's biology, microbial pathogens, predators and parasites, and management. We compiled this bibliography for the use of entomologists, veterinary pathologists, and scientists in related fields who are studying pathology of the ETC/equine interaction, as well as persons concerned with managing ETC on horse farms, in the hope of saving others' time and avoiding duplication of effort. The 103 citations are organized into particular subject areas. Our goal is to expedite ongoing and future studies by organizing relevant citations in a format where they will be readily available to MRLS researchers.

Introduction

This bibliography covers published research on biology and control of the eastern tent caterpillar (ETC), *Malacosoma americanum* (F.) (Lepidoptera: Lasiocampidae) that may be relevant to management of Mare Reproductive Loss Syndrome. Farm surveys and experimental induction of MRLS by directed exposure of pregnant mares to ETC in field plots or by gastric administration all suggest that ETC somehow is involved with the disease. This revelation that ETC may be more than a nuisance tree pest is spurring interest in its microbial pathogens, dispersal behavior, spatial distribution, population biology, and management. Fitzgerald (1995) also cites many of the references compiled here; however, there they are presented alphabetically and intermixed with citations to species other than *M. americanum.* We updated the list of citations to ETC, organized it by subject matter, and streamlined it to help direct MRLS researchers, pest management advisors, and farm managers to the relevant literature.

Materials and Methods

Electronic databases (e.g., Agricola, Agris, Biological Abstracts) accessed through ERL WebSPIRS version 5.02, as well as the search engines Excite® and Google®, were used to find citations related to ETC. Searches were performed on the key words "eastern tent caterpillar" and *Malacosoma americanum.* Reference lists in two books devoted to tent caterpillars (Dethier 1980, Fitzgerald 1995) also were examined. Besides those books, the 103 citations we list are organized into nine categories: behavior, general biology, genetics and evolution, management, nutritional ecology, pathogens, pheromones, physiology and seasonal ecology, and predators and parasitoids. We made no attempt to include State and Federal publications that only reiterate general biology and control recommendations. Articles concerning ETC published in this proceedings also are not listed.

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Mare Reproductive Loss Syndrome Suggested Reading List

Gracie Hale

FOLLOWING IS A SUGGESTED READING LIST OF ARTICLES, papers, and other publications relevant to Mare Reproductive Loss Syndrome. The list is arranged in chronological order, with each year's publications additionally arranged alphabetically by author.

1. Bryans, J. T. Report on early fetal losses: Department of Veterinary Science, University of Kentucky. 1981.
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Morris Library, Maxwell H. Gluck Equine Research Center, College of Agriculture, University of Kentucky, Lexington, Kentucky. The Morris Library maintains and updates this list, as well as retaining copies of the various publications. For further information, to request that an article be added to the list, or to receive updates of the list, please contact Gracie Hale, Morris Library, Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546. Telephone: 859-257-1192 Fax: 859-257-8542, Email: ghale@uky.edu.

Internal Medicine Veterinary Medical Forum.; Dallas, Tex. 2002. Available at: <http://www.vin.com/Members/Proceedings>.

19. Dwyer, R. M.; Garber, L.; Traub-Dargatz, J.; Meade, B.; Pavlick, M., and Walker, J. An epidemiological investigation of mare reproductive loss syndrome: breaking ground on a new disease. Society for Veterinary Epidemiology and Preventive Medicine. 2002; 44-47.
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