Invited Review: The role of caterpillars in mare reproductive loss syndrome: A model for environmental causes of abortion

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ABSTRACT: A new abortigenic disease, now known as mare reproductive loss syndrome (MRLS), significantly affected the horse industry in the Ohio River Valley of the United States in late April and early May of 2001 and 2002. In 2001, approximately 25% of all pregnant mares aborted within several weeks (over 3,000 mares lost pregnancies), and abortion rates exceeded 60% on some farms. Mare reproductive loss syndrome struck hard and without warning, it was caused by something in the environment, it was not transmitted between animals, and it was not associated with any known abortigenic agent or disease. These experiments demonstrated that horses will inadvertently consume Eastern tent caterpillars (ETC) when the insects are present in the pasture or other feedstuffs, and MRLS-type abortions were induced in experimental animals (mares and pigs) by mixing ETC with the feed of the animals. Eastern tent caterpillars are hirsute (hairy) caterpillars, and the only part of the caterpillar that caused MRLS abortions was the cuticle. The experiments revealed that the setae (hairs) embed into the submucosa of the alimentary tract creating microgranulomatous lesions. It is hypothesized that the alimentary tract lesions allow bacteria from the alimentary tract of the mare, principally streptococci, actinobacilli, and to a lesser extent enterococci, to invade the circulatory system of the mare. The bacteria then establish infections in tissues where the immune surveillance of the mare is reduced, such as the fetus and placenta. Fetal and placental fluid bacterial infections lead to fetal death and abortion characteristic of MRLS. Inadvertent ingestion of ETC by pregnant mares causes MRLS. Currently the only known means to prevent MRLS is to avoid exposure of horses, particularly pregnant mares, to ETC and probably most hirsute caterpillars.

Key words: abortion, caterpillar, equine, fetus, mare reproductive loss syndrome, reproduction

INTRODUCTION

In 2001, approximately 25% of broodmares in central Kentucky aborted within several weeks (over 3,000 mares lost pregnancies), and abortion rates exceeded 60% on some farms. The abortion storm was characterized by early (~40 to 150 d gestation; d Ga) and late (near-term, where gestation averages ~340 d) fetal losses, and submissions of late-term abortions or stillbirths to the University of Kentucky Livestock Disease Diagnostic Center (LDDC) increased from a normal rate of approximately 5 per day to more than 30 per day (Figure 1). The abortigenic disease, now known as mare reproductive loss syndrome (MRLS), struck hard and without warning. The disease was caused by something in the environment, was not transmitted between animals, and was not associated with any known abortigenic agent or disease. It was a newly recognized disease that cost the state of Kentucky ~$330 million in 2001 alone (Thalheimer and Lawrence, 2001).

The first reports in May 2001 came from veterinarians who stated that early fetal losses were characterized by hyperechogenic amniotic or allantoic fluids or
both surrounding a dead fetus, or a live fetus with a slow heart rate that died and was expelled several days later (Morehead et al., 2002; Riddle, 2003). Primary pathological findings in late-term MRLS fetuses were funisitis of the amniotic umbilical cord, pneumonia, bacterial infections, and hemorrhages (Donahue et al., 2003; Williams et al., 2003). The first epidemiological survey was conducted in the summer of 2001 and revealed a temporal correlation between MRLS and presence of Eastern tent caterpillars (ETC; *Malacosoma americanum*), wild black cherry trees (*Prunus serotina*), waterfowl, and feeding hay off of the ground in horse pastures where mares aborted (Dwyer et al., 2003), submitted to the University of Kentucky Livestock Disease Diagnostic Center each day over the time period shown. The figure is reproduced in part from Webb et al. (2004).

**EXPERIMENTAL ANIMALS**

All animals had water available ad libitum and were used with the approval of the University of Kentucky Institutional Animal Care and Use Committee.

Pregnant mares used in these experiments were between 40 and ~200 d Ga. When not on individual treatment plots or being fed individually, they were housed communally on grass paddocks and supplemented with hay as needed. Forages in the paddocks were principally Kentucky bluegrass (*Poa pratensis*, ~25 to 55%), orchardgrass (*Dactylis glomerata*, ~5 to 25%), white clover (*Trifolium repens, ~2 to 15%*, *Festuca arundinacea, ~0 to 20%*), and weeds (~1 to 15%), and varied somewhat by year and by season. Pregnancies were monitored by manual palpation and real-time ultrasonography weekly during the first experiment, and daily or multiple times daily in all subsequent experiments, beginning before d 1 of treatment and continuing for at least 30 d after the final treatment. Aborted fetuses and placentas, uterine culture swabs, and biopsies obtained immediately after abortion, as well as blood samples from the mares (10 mL via jugular venipuncture), were taken to the LDDC for necropsy, histopathology, bacteriology, serology, and virology. Nonpregnant mares were housed communally on grass pastures. Pregnant gilts (*Sus scrofa*), 54 to 59 d Ga at the beginning of the experiment, were housed individually in gestation stalls, and treatments were added to their normal gestation ration daily. All laboratory personnel were blinded to animal treatments throughout all of the experiments. The sonographers and animal handlers were blinded to treatments in the horse experiments but not in the experiment with pregnant gilts.

### EASTERN TENT CATERPILLARS CAUSE MRLS

**ETC in Pastures Where Mares Graze Cause Abortions**

Details of the first 4 experiments can be found elsewhere (McDowell et al., 2003; Webb et al., 2003, 2004) and are only briefly described here. That both wild black cherry trees and ETC were implicated in the original epidemiological study (Dwyer et al., 2003) is not surprising because wild black cherry trees are the preferred ovipositional host tree for ETC, and ETC larvae develop most successfully on these trees (Fitzgerald, 1995). However, ETC larvae were present in the Ohio River Valley in record numbers in the spring of 2001. Therefore, the initial 2 experiments, conducted May through July 2002, were designed to test the hypothesis that ETC would cause fetal loss in pregnant mares. The design was to mimic the natural exposure that occurred during the MRLS outbreak of 2001 as closely as possible, but in a controlled research environment. In the first experiment, for 20 d, 6 h/d, pregnant mares (~50 to 200 d Ga) were housed individually in 4.8 m × 4.8 m pens placed on grass pasture. Pens were moved to fresh pasture plots each day such that horses were grazing fresh pasture each day, and experimental materials were placed into each pen each day. Pregnant mares were exposed to live ETC larvae and nest materials [nest materials included the nest silk, frass (caterpillar excrement), and cast-off cuticle from molting larvae; n = 10 mares] or nest materials only (n = 9 mares), whereas control mares (n = 10) had no experimental materials placed in their pens. Those control mares were handled identically to the mares on experimental treatments, but attempts were made to minimize their exposure to ETC or ETC materials. A physical barrier meant to contain the caterpillar larvae was created from a 7.6-cm polyvinylchloride pipe, which was cut in
half and placed around each treatment block. However, sampling of the plots revealed that ETC larvae were not contained in their respective treatment plots as originally planned, and larvae were observed in some of the nest material-only plots as well as some of the control plots, thus invalidating between-group comparisons. In this first experiment 18 of the 29 mares aborted fetuses with signs consistent with MRLS.

The second experiment was of similar design, except that mares (~50 to 200 d Ga) were housed individually in the pens 6 h/d for 10 d. The caterpillar barriers were improved for Exp. 2, including the same physical barriers as well as an insecticide barrier applied just to the outside of the polyvinylchloride pipes. In addition, it was shown that ETC frass supported growth of various fungi, which in turn had been reported to have adverse health effects in other species (Newman, 2003). Therefore, to differentiate effects of insect larvae or other nest materials from that of insect frass, frass was separated from other nest materials by sifting through a wire screen, and insect larvae were not fed for approximately 2 wk before the study so that they would deliver little or no frass to the treatment plots. Thus in Exp. 2, pregnant mares were exposed to nonfed ETC larvae, frass only, or pasture only (no ETC materials added to the pasture plots, controls).

The only pregnancy losses were in mares directly exposed to live ETC larvae (Table 1). Daily ultrasound examinations of all mares showed that mares exposed to the caterpillars and that subsequently aborted showed a progressing increase in the echotexture of fetal fluids beginning 1 to 4 d before fetal loss, which was the only sign of impending fetal death. Serum concentrations of estrogens and progestins declined associated with abortion, but the decline was after fetal death. There were no similar changes in any mare that did not abort. Bacterial isolates from the aborted fetuses included streptococci, enterococci, and serratiae. Signs of abortion were consistent with MRLS as it had been reported by field veterinarians in 2001 (e.g., few to no premonitory signs of impending abortion in the mares, increased echogenicity of fetal fluids before fetal death).

Table 1. Fetal losses for mares fed Eastern tent caterpillars (ETC), ETC materials, or gypsy moth caterpillars (GMC) in each experiment

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Fetal loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETC cause mare abortions</td>
<td></td>
</tr>
<tr>
<td>Treatment (50 g of insect larvae per mare each day for 10 d)</td>
<td></td>
</tr>
<tr>
<td>Non-fed ETC</td>
<td>3/7* (10 to 14)</td>
</tr>
<tr>
<td>ETC frass</td>
<td>0/6</td>
</tr>
<tr>
<td>No ETC products</td>
<td>0/7</td>
</tr>
<tr>
<td>Direct feeding of insect larvae</td>
<td></td>
</tr>
<tr>
<td>Treatment (50 g of insect larvae per mare each day for 8 d)</td>
<td></td>
</tr>
<tr>
<td>Frozen ETC</td>
<td>3/5* (11 to 28)</td>
</tr>
<tr>
<td>Frozen/autoclaved ETC</td>
<td>0/5</td>
</tr>
<tr>
<td>Frozen GMC</td>
<td>1/4 (16)</td>
</tr>
<tr>
<td>ETC dissection and filtration</td>
<td></td>
</tr>
<tr>
<td>Treatment (50 or 75 g of insect larvae per mare each day for 10 d)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0/5</td>
</tr>
<tr>
<td>ETC (positive control)</td>
<td>5/5* (4 to 14)</td>
</tr>
<tr>
<td>ETC dissection</td>
<td></td>
</tr>
<tr>
<td>Cuticle</td>
<td>3/5* (3 to 9)</td>
</tr>
<tr>
<td>Gut</td>
<td>0/5</td>
</tr>
<tr>
<td>Internal tissues</td>
<td>0/5</td>
</tr>
<tr>
<td>ETC homogenization and filtration</td>
<td></td>
</tr>
<tr>
<td>Solids and retentate</td>
<td>1/5** (31)</td>
</tr>
<tr>
<td>Filtrate</td>
<td>0/5</td>
</tr>
<tr>
<td>Cuticular composition and structure</td>
<td></td>
</tr>
<tr>
<td>Treatment (50 or 75 g of insect larvae per mare each day for 10 d)</td>
<td></td>
</tr>
<tr>
<td>Corn oil (negative control)</td>
<td>0/5</td>
</tr>
<tr>
<td>ETC cuticle (positive control)</td>
<td>3/5* (8 to 32)</td>
</tr>
<tr>
<td>ETC cuticle powdered</td>
<td>1/5** (31)</td>
</tr>
<tr>
<td>ETC cuticle after organic solvent extraction</td>
<td>2/5** (16)</td>
</tr>
<tr>
<td>Corn oil containing organic solvent extraction of cuticle</td>
<td>0/5</td>
</tr>
</tbody>
</table>

1Mares were between 40 and ~200 d of gestation the beginning of each experiment.
2Number of abortions/number of mares in each treatment group.
3The range in days from first exposure to ETC to abortion is shown in parentheses.
4When larvae were dissected, homogenized, extracted, or powdered, each treatment consisted of material from 75 g of insects to account for procedural losses.

*P < 0.05.
**P > 0.05. Although not statistically different from controls, abortions in these mares were biologically characteristic of mare reproductive loss syndrome (e.g., increased echogenicity of fetal fluids; types of bacteria isolated of the fetuses after abortion; no premonitory signs of abortion in mares).
and abortion, and presence of bacteria in fetal fluids and fetal tissues).

These studies provided the first experimental evidence that ETC can induce pregnancy loss in horses and were the first to reproduce the syndrome under experimental conditions. Results of the first 2 experiments were released to the public in the spring of 2002 before caterpillar larvae were present in large numbers on horse farms. In 2002, equine abortions and foal losses were approximately one-third that of 2001, in part due to decreased numbers of caterpillars. Additionally, this initial experimental evidence demonstrating that live ETC could cause MRLS led farm managers to take measures to reduce the exposure of pregnant mares to ETC in 2002.

The Abortigenic Activity Is Stable to Freezing and Thawing but Is Destroyed by Autoclaving, and Pasture Grass Is Not a Necessary Component of the Disease

The objectives of Exp. 3 were to begin to understand whether the abortigenic agent was biological or toxicological, whether an association between ETC and pasture grasses was a necessary component of MRLS, and whether the abortigenic activity was specific to ETC or potentially characteristic of other hirsute caterpillars. The hypotheses tested were 1) direct feeding of ETC causes MRLS-type abortions in pregnant mares, 2) the abortigenic activity is stable to freezing and autoclaving, and 3) the abortigenic activity is associated with other hirsute caterpillars. Eastern tent caterpillar larvae were collected and stored frozen at −80°C, and a portion of those were also autoclaved and refrozen before use. Frozen gypsy moth caterpillar larvae (GMC, *Lymantria dispar*) were supplied by M. Shapiro (USDA, Beltsville, MD).

Treatments were mixed with ~500 g of normal grain diet and fed to each mare (~45 to 100 d Ga) individually from feed buckets. Increased echotexture of fetal fluids and subsequent fetal death occurred in all ETC-fed mares that aborted and in none of the mares that did not abort (Table 1). Bacteria most frequently isolated from the fetuses were pure or near-pure cultures of non-β-hemolytic streptococci or actinobacilli or both; however, no streptococci or actinobacilli were isolated from ETC larvae.

One of 4 mares fed frozen GMC larvae aborted. Fetal loss in that mare was not characteristic of MRLS; the fetus did not grow normally during the course of the experiment and fetal death was not accompanied by an increase in echotexture of the fetal fluids. *Streptococcus equisimilis* (β hemolytic) were isolated from the placenta and umbilical cord of the fetus from the GMC-fed mare, but cultures of the lung and liver produced no significant bacteria. Thus, in this experiment, although the single abortion from a mare receiving GMC was not typical of MRLS, the results of feeding a different hirsute caterpillar were deemed inconclusive.

These experiments did show that the abortigenic activity was stable to freezing and thawing, labile to autoclaving, and was not associated with pasture grass. In addition, extensive monitoring of local horse farms showed no correlations in pasture composition, fertilization, or other management practices, between pastures where mares were affected by MRLS and pastures where mares were not affected by MRLS (J. C. Hennig, Cooperative Extension, University of Kentucky, Lexington, personal communication).

The Abortigenic Activity Is Associated Only with the Cuticle of the Caterpillar

To further describe the nature of the abortigenic activity, 2 additional experiments were conducted between August 2002 and August 2003. The aims were to determine if the abortigenic activity was localized to any particular portion of the insect larvae, if it were extractable, or if it would pass through or be retained by a biological filter. The hypotheses tested were as follows: 1) the abortigenic activity is associated with a particular physical component of the caterpillar, 2) the abortigenic activity can be extracted in an aqueous solution, 3) the abortigenic activity can be extracted in an organic solution, and 4) disrupting the physical structure of the cuticle (exoskeleton) alters the abortigenic activity.

ETC Dissection and Filtration. Thirty-five pregnant mares (~55 to 120 d Ga) were randomly assigned to 1 of 7 treatments as shown in Table 1. The experimental treatments were of 2 types: dissection and saline extraction/filtration. The first component of this experiment entailed dissection of insect larvae into 3 components: gut (foregut, midgut, and hindgut), internal tissues (fat body, hemolymph, salivary glands, Malpighian tubules, muscle, and epidermis), and cuticle. Positive and negative control groups received frozen (intact) ETC or saline, respectively. Eastern tent caterpillar larvae feed on the leaves of black cherry trees, and the leaves are known to contain cyanogenic compounds that may be fetotoxic (Poppenga, 2003). Thus, the gut fraction would be expected to contain any cyanogenic activity from macerated cherry tree leaves. The internal tissues would contain ETC-specific bacteria, viruses, and microsporidia, as well as other pathogens that potentially could adversely affect the health of animals consuming the insect larvae. The cuticle is composed of relatively inert chitin material and was not anticipated to have adverse effects in the mares or their fetuses. In the extraction and filtration component of this experiment ETC larvae were homogenized in PBS, filtered through a nylon mesh, centrifuged twice to remove solids (16,000 × g and then 22,000 × g, 15 min each at room temperature), and finally filtered through a 0.45-micron filter. This resulted in a filtrate fraction and a retentate fraction.

All treatments were mixed into the grain normally fed to the mares, and mares were fed individually from...
feed buckets. Fetal losses occurred only in mares receiving intact ETC, ETC cuticle, and the retentate fraction, which contained pieces of cuticle after homogenization.

**Cuticular Composition and Structure.** Twenty-five pregnant mares (~60 to 150 d Ga) were randomly assigned to 1 of 5 treatments as shown in Table 1. Cuticles of ETC were collected by dissection, and a portion of these were crushed to a powder with a mortar and pestle over liquid nitrogen. Lipids were extracted from ETC cuticle with methylene chloride, which was then evaporated through corn oil with a rotating evaporator, transferring the extracted lipids to the corn oil. The corn oil was then added to the grain fed to each mare. An additional treatment consisted of feeding ETC cuticle to mares after the cuticle had gone through the organic extraction process. Positive and negative control groups of mares were fed ETC cuticle and corn oil, respectively. The control corn oil was mixed with methylene chloride, and then the solvent was removed with a rotating evaporator. Once again, the only treatments that caused fetal losses included insect cuticle, and the abortigenic activity was reduced when the physical structure of the cuticle was disrupted. These experiments demonstrated that the abortigenic activity was associated only with the cuticle of the caterpillar, it could not be extracted with either an aqueous or organic solution, and it was reduced when the cuticle of the caterpillar was disrupted.

**ETC CAUSE FETAL LOSS IN PIGS, AND THE SETAE CREATE LESIONS IN THE ALIMENTARY TRACT OF ANIMALS THAT INGEST THE CATERPILLARS**

During the natural MRLS outbreaks of 2001 and 2002, fetal deaths and abortions were reported only in horses. For this experiment pigs were selected as test animals because pigs and horses are nonruminants and have similar (epitheliochorial) types of placentation. To test whether ETC could cause abortions in an animal other than the horse, 10 pregnant gilts (54 to 59 d Ga at time of first feeding) received intact ETC (previously frozen) in their daily gestation diet or received their daily gestation diet without ETC. Gilts were paired according to gestation dates, with 1 ETC-fed gilt and 1 control gilt in each pair.

Two of 5 gilts fed ETC aborted their entire litters (Table 2). Those 2 gilts and their paired control gilts were euthanized within 3 d of fetal loss. The remaining 3 ETC-fed gilts and their control pairs were still pregnant at the time of euthanasia, 29 d after the first ETC treatment. *Streptococcus bovis* was isolated from aborted fetuses in 1 litter, and an α-hemolytic *Streptococcus* species was isolated from the aborted fetuses of the other litter. Of particular interest was the identification of caterpillar setae and setal fragments in the alimentary tract of all gilts receiving ETC but in none of the control gilts. The ETC setae were embedded into the submucosal lining throughout the alimentary tract and were surrounded by microgranulomatous lesions. That caterpillar setae embed into the alimentary tract causing microgranulomatous lesions when caterpillar larvae

### Table 2. Fetal losses in pregnant gilts fed Eastern tent caterpillars (ETC) for 10 d

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Fetal loss&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETC cause abortions in pregnant gilts</td>
<td></td>
</tr>
<tr>
<td>Treatment (40 g of insect larvae per gilt each day for 10 d)</td>
<td></td>
</tr>
<tr>
<td>ETC</td>
<td>2/5 (13 to 16)&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>No insect exposure</td>
<td>0/5</td>
</tr>
</tbody>
</table>

<sup>1</sup>Gilts were between 54 and 59 d of gestation at first ETC feeding.

<sup>2</sup>Number of abortions/number of gilts in each treatment group.

<sup>3</sup>The range in days from first exposure to ETC to abortion is shown in parentheses. Microgranulomatous lesions were observed in histological sections of the alimentary tracts in all gilts receiving ETC but in none of the control gilts.
are added to the grain of horses was subsequently confirmed in pregnant and nonpregnant mares (Figure 2).

**Immune Responses to ETC or Setae**

To test the hypothesis that ETC setae alter the immune response of the mare, 2 additional experiments were performed. In the first experiment, mares (n = 6 nonpregnant and n = 11 pregnant, ~40 to 100 d Ga) were treated with ETC setae that had been ground to a powder, resuspended in saline, and subjected to 5 KGy of ionizing radiation. Nonpregnant (NP) and pregnant (Preg) mares were used. There was an effect of pregnancy when PWM was injected, regardless of whether setae were added. There was no effect of pregnancy when saline was injected, regardless of whether setae were added. Setae in saline resulted in lesions more typical of foreign body reactions (Rxns), whereas setae in PWM resulted in lesions more typical of hypersensitivity reactions.

![Figure 3](image-url)

**Figure 3.** Skin biopsy scores (means ± SEM) for mares receiving intradermal injections of saline or pokeweed mitogen (PWM), each containing 2 or 20 µg of pulverized Eastern tent caterpillar setae. Biopsy specimens were subjectively scored from 0 (no observed inflammatory cell infiltration; i.e., normal) to 4 (a marked, severe inflammatory reaction). Nonpregnant (NP) and pregnant (Preg) mares were used. There was an effect of pregnancy when PWM was injected, regardless of whether setae were added. There was no effect of pregnancy when saline was injected, regardless of whether setae were added. Setae in saline resulted in lesions more typical of foreign body reactions (Rxns), whereas setae in PWM resulted in lesions more typical of hypersensitivity reactions.

Results from the above 2 experiments, although not exhaustive, led us to conclude that ETC setae caused an immune response in mares similar to a foreign body reaction rather than a hypersensitivity reaction, and direct ingestion of ETC does not alter inflammatory cytokine mRNA levels. Therefore, it is unlikely that a direct immune response to ETC or ETC setae are major factors in MRLS.

**DISCUSSION**

The most common postmortem finding in MRLS, from field cases and from experimentally induced MRLS, is fetal colonization by bacteria, which are almost always non-β-hemolytic streptococci, actinobacilli, or enterococci (Donahue et al., 2003). Phenotypic characterization has revealed that *Streptococcus criceti* was the most frequently isolated species (J. M. Donahue, personal observations), and *Actinobacillus equuli* using calipers and skin biopsies were obtained at each treatment site. The biopsy samples were fixed, stained, sectioned for microscopic examination, and subjectively scored on a grading scale of 0 (no observed inflammatory cell infiltration, i.e., normal) to 4 (a marked, severe, inflammatory reaction).

There were no differences between mares that had known prior ETC exposure and those that had not, so data for those groups were combined. All treatments containing PWM resulted in increased ($P < 0.0001$) induration sizes compared with any treatment not containing PWM, regardless of whether ETC setae were added. Biopsy scores were greater from sites receiving PWM than sites not receiving PWM, regardless of whether setae were added (Figure 3). Biopsies containing inflammatory cells typically had infiltrates of neutrophils, macrophages, and eosinophils with degrees of interstitial edema. In treatments that contained PWM, the inflammation was more focally diffuse in distribution, whereas the reaction to setae tended to be more concentrated and had microscopically visible setal fragments and some necrosis of the tissue and inflammatory cells surrounding setae.

To determine if exposing mares to ETC in their feed caused a change in inflammatory cytokine mRNA production, 12 nonpregnant mares were fed grain alone (n = 6) or grain containing ETC (n = 6; 50 g of ETC/mare) once daily for 8 d. Blood samples (2.5 mL via jugular venipuncture) were collected on d 0, 3, 5, 7, 9, 11, and 13 relative to caterpillar feeding. Blood was collected into PreAnalytiX PAXgene Blood RNA tubes (vacuum tubes containing 6.9 mL of Hemogard, QIAGEN, Valencia, CA) with subsequent isolation of RNA. The corresponding cDNA was analyzed by real-time reverse transcription-PCR for the cytokines IL-1, -6, and -10, interferon-γ, and tumor necrosis factor. Although cytokine RNA levels occasionally increased or decreased in some animals, there were no treatment × day interactions for any cytokine tested ($P > 0.5$).

Results from the above 2 experiments, although not exhaustive, led us to conclude that ETC setae caused an immune response in mares similar to a foreign body reaction rather than a hypersensitivity reaction, and direct ingestion of ETC does not alter inflammatory cytokine mRNA levels. Therefore, it is unlikely that a direct immune response to ETC or ETC setae are major factors in MRLS.
Caterpillars and mare reproductive loss syndrome

was the most frequently isolated actinobacillus species (Donalhue et al., 2006). *Streptococcus* species and *Actinobacillus* species isolated from recovered fetuses and pericardial fluid samples of horses affected by MRLS were identical to those found in the oral cavity and alimentary tract of healthy horses, and these bacteria were not cultured from ETC larvae (Donalhue et al., 2006). It is not uncommon that bacterial infections are associated with fetal or foal loss in broodmares, due at least in part to the fact that mares have extremely little passive transmission of antibodies to the foal in utero (Jeffcott, 1972; Sheoran et al., 2000).

Mare reproductive loss syndrome can be considered to be similar to oropharyngeal insults in humans that can cause a transient low-grade bacteremia. In humans, nonhemolytic streptococci represent a large proportion of the commensal bacteria present on oropharyngeal surfaces (Hardy and Bowen, 1974; Fransen et al., 1991). These bacteria have been shown in several studies to enter the bloodstream after trauma to oral tissues (Ness and Perkins, 1980; Coulter et al., 1990). Bacteremic infections associated with oropharyngeal insult include endocarditis, septic arthritis, and prosthetic joint infection (Fitzgerald et al., 1982; Bayliss et al., 1983; Davies et al., 1988). In addition, in humans and mice, periodontal infections are associated with low birth weight, preterm delivery, and fetal infection (Offenbacher et al., 1996; Li et al., 2000; Madianos et al., 2001). In the natural farm occurrence of MRLS, over 3,000 mares lost pregnancies. There were also increased numbers of cases of pericarditis (pericardial fluid from 13 clinical cases and 22 horses were submitted to LDDC; Bolin et al., 2003) and unilateral endophthalmitis (24 cases reported by 1 veterinary practice; Latimer, 2003). Horses on commercial farms were likely exposed to ETC most of each day for several weeks, whereas in our experiments mares were exposed to the insects in their pasture plots for several hours a day or had insect or insect components mixed directly into their feed. Whereas it is likely that ETC exposure caused the pericarditis and endophthalmitis reported by farm veterinarians, with the smaller number of experimental animals in these studies, and the limited contact time with caterpillar larvae, it was not surprising that no cases of pericarditis or endophthalmitis occurred under these experimental conditions.

Mare reproductive loss syndrome is not a disease solely found in the Ohio River Valley of the United States. Indeed, MRLS has been reported in Florida (Zimmel, 2005), and more recently, an identical syndrome called equine amnionitis and fetal loss was reported in Australia where field cases of mare abortions were associated with an abundance of processionary caterpillars (*Ochragaster lunifer*; Cawdell-Smith et al., 2007). Those abortions mimicked the signs of MRLS and the syndrome was induced in pregnant mares experimentally by administering macerated caterpillars via a nasogastric tube (Cawdell-Smith et al., 2007). Thus, despite our inconclusive studies with GMC, ETC are not the only hirsute caterpillar that can cause MRLS. To date, natural or experimental occurrences of MRLS have only been associated with hirsute caterpillars, and the only portion of the caterpillar demonstrated to cause MRLS is the setae-containing cuticle. Indeed, some initial research on MRLS was targeted toward agents potentially associated with the ETC gastrointestinal tract (gut fraction) such as cyanide (Harkins et al., 2003) or mandelonitrile (Camargo et al., 2003), or noninsect agents such as mycotoxins (Newman, 2003), fescue toxicosis (Schultz and Bush, 2003), and unusual weather patterns (Priddy et al., 2003). To date, no agent other than hirsute caterpillars, specifically the setae-containing cuticle, has been demonstrated to reproduce MRLS.

The MRLS abortion storms of 2001 and 2002 were characterized by early and late fetal losses. The majority of broodmares in central Kentucky are Thoroughbreds, and their breeding season is controlled such that in late April and early May of any given year the vast majority of pregnant mares were either bred earlier in the year and are less than ~100 d Ga or were bred the previous year and are late in gestation awaiting foaling. It is now clear that MRLS can occur in pregnant mares anytime after ~40 d Ga. Interestingly, mares less than ~40 d Ga were not affected in the natural farm cases and therefore were not used in these MRLS experiments. Interdigitating microvilli of the trophoblast and uterine epithelium begins by ~38 d Ga, and microvilli become macrovilli, which are precursors of the microcotyledons, around 45 d Ga (Ginther, 1992). Thus, the first real attachment of the fetal trophoblast to the uterine endometrium occurs at the same time pregnant mares become susceptible to MRLS.

**Conclusions**

The experiments described in this review led to the hypothesis that ETC setae penetrate the alimentary tract lining of animals that ingest them, causing a subclinical bacteremia. Bacteria, primarily non-β-hemolytic streptococci and, to a lesser extent actinobacilli and enterococci, establish infections in tissues where the immune surveillance of the mare is reduced, such as the fetus and fetal fluids. Fetal and fetal fluid infections lead to fetal losses characteristic of MRLS, and MRLS can be considered to be similar to oropharyngeal insults in humans that can cause a transient low grade bacteremia, low birth weights, preterm delivery, and fetal infections.

The entire Proceedings of the First Workshop on Mare Reproductive Loss Syndrome, 2003, can be found online at http://www.ca.uky.edu/agc/pubs/sr/sr2003-1/sr2003-1.htm (last accessed December 12, 2009). The Veterinary Science Department at the University of Kentucky maintains an MRLS Web site at http://www.ca.uky.edu/gluck/MRLSindex.asp, where updates and
news releases are posted. Videos of horses inadvertently consuming ETC in their pasture or other feedstuffs can be accessed through the above Web site as well.

LITERATURE CITED


