Evaluation of early fetal loss induced by gavage with eastern tent caterpillars in pregnant mares

William V. Bernard, DVM, DACVIM; Michelle M. LeBlanc, DVM, DACT;
Bruce A. Webb, PhD; Arnold J. Stromberg, PhD

Objective—To determine whether gavage of pregnant mares (housed without access to pasture) with starved eastern tent caterpillars (ETCs) or their excreta is associated with early fetal loss (EFL), panophthalmitis, or pericarditis.

Design—Randomized clinical trial.

Animals—15 mares.

Procedure—15 mares with fetuses from 40 to 80 days of gestation (dGa) were randomly assigned to 1 of 3 groups and received 2.5 g of ETC excreta, 50 g of starved ETCs, or 500 mL of water, respectively, once daily for 10 days. Mares were housed in box stalls, walked twice daily, and not allowed access to pasture for 12 days before or during the 21-day trial.

Results—4 of 5 mares gavaged with starved ETCs (group 2) aborted on trial days 8 (2 mares), 10, and 13. No control mares or mares that received excreta aborted. Differences between the ETC group and other groups were significant. Abortion occurred on 49, 64, 70, and 96 dGa. Allantoic fluids became hyperechoic the day before or the day of fetal death. Alpha streptococci were recovered from 1 fetus and *Serratia marcescens* from 3 fetuses. Neither panophthalmitis nor pericarditis was seen. The abortifacient component of the ETCs was not elucidated.

Conclusions and Clinical Relevance—These findings suggest that mares with fetuses from 40 to 120 days of gestation should not be exposed to ETCs because they may induce abortion. (*J Am Vet Med Assoc* 2004; 225:717–721)

In late April and early May of 2001, an unusual syndrome affected pregnant mares in north central Kentucky, southern Ohio, Indiana, and Illinois. Veterinarians reported that an excessive number of previously pregnant mares were found to have either no fetus or a dead or dying fetus at the reproductive ultrasound examination conducted at 60 to 70 days of gestation. Affected mares typically had no clinical signs. A small percentage (estimated at < 5%) had mild signs of colic, abdominal straining, or low-grade fever ([38.33° to 38.61°C] [101° to 101.5°F]) 1 to 3 days before **early fetal loss (EFL)** occurred. Alpha streptococci were most commonly isolated from aborted fetuses. Oddly, the syndrome appeared to affect only mares with fetuses of gestation time > 35 days.[1] By June of 2001, approximately 2,000 EFLs were reported.[2] Early fetal losses varied among farms, with reported incidences of 5% to 35%.[3] Concurrently, there was a dramatic increase in the number of late-term aborted fetuses submitted to the University of Kentucky Livestock Disease Diagnostic Center.[2] Local equine hospitals reported a marked increase in the incidence of sick newborn foals,[4] pericarditis,[5] and unilateral uveitis[6] in yearlings and adults. No infectious or contagious agents could be identified as the cause.[1] The simultaneous EFLs and late-term fetal losses were subsequently named **mare reproductive loss syndrome (MRLS)**. In 2001, losses to the Thoroughbred industry alone were estimated at $336 million.[7]

An epidemiologic survey conducted by the University of Kentucky identified 4 factors associated with increased incidence of MRLS, including breeding date in February 2001, moderate to high concentration of **eastern tent caterpillars (ETCs** [*Malacosoma americanum*]) in areas with mares, wild cherry trees around pastures, and having more than 50 mares on the farm.[8] Two factors were associated with low incidence or no incidence of MRLS: absence of ETCs and feeding hay to mares in pasture.[8] A subsequent case-control study of EFLs found 5 factors that increase the risk of EFL, including feeding hay in pasture during the 4-week period prior to abortion, a greater than usual amount of white clover in pasture during the 4-week period prior to abortion (and during the spring of 2001 relative to the spring of 2000), a larger than usual population of ETCs in pastures during 2001, abortion during the previous 5 years, and elk or deer being seen at the premises during the preceding 12 months.[9]

A trial that exposed pregnant mares to ETCs and their excreta (frass) was undertaken.[10] Pregnant mares housed on pasture were exposed to either ETCs with excreta or excreta alone. Ten mares housed under identical conditions were maintained as control mares. Excreta and ETCs with excreta were spread on pasture

From the Rood and Riddle Equine Hospital, PO Box 12070, Lexington, KY 40511 (Bernard, LeBlanc); and the Departments of Entomology (Webb) and Statistics (Stromberg), College of Arts and Sciences, University of Kentucky, Lexington, KY 40506-0027.

Supported by The Grayson Jockey Club, Rood and Riddle Equine Hospital, TaylorMade Farm, Ernie Paragallo, Fasig-Tipton, the Kentucky Thoroughbred Farm Managers Club, and USDA:ARS 58-6401-2-0025.

Publication 03-08-099 of the University of Kentucky Agricultural Experiment Station.

Presented at the 1st Workshop on Mare Reproductive Loss Syndrome (MRLS), Lexington, Ky, August 2002.

The authors thank Steve Vargas, Elizabeth McCutcheon, Tammy Parker, Barbara Sheerin, and Drs. Johanna Reimer, Claire Latimer, and Bart Barber for technical assistance.

Address correspondence to Dr. Bernard.

to which mares were exposed for 6 h/d. Most mares in the groups exposed to ETCs with excreta (7/10) or excreta alone (7/9) aborted. Four of 10 mares in the control group also aborted, and sampling of control pastures for larvae revealed that ETC larvae were present on all pastures because they had escaped from the pastures to which ETCs had been experimentally added. The study reported here was designed to complement the previous study by removing the mares from exposure to grass and more strictly control the administration of ETCs and excreta. It was hypothesized that mares gavaged with either starved ETCs or their excreta would abort.

Materials and Methods

Mares—Fifteen healthy mixed-breed pregnant mares with fetuses from 40 to 80 days of gestation on day 1 of the study were used. Mares were selected from an initial group of 42 mares obtained from commercial nurse mare operations in northern Kentucky. Eighteen of the 42 mares were initially selected and placed in quarantine for a minimum of 12 days. Mares had fetuses (gestation from 28 to 65 days) on the first day of quarantine, unremarkable results of physical examinations, body condition score of 5 to 7.5, and normal external genitalia. Pregnancy was confirmed by transrectal ultrasonography. Previous reproductive history of mares was unknown. Breeding dates were known for all mares with fetuses > 50 days of gestation. Gestational age of the fetus was approximated in 7 mares by measuring the size of the embryonic vesicle and the position of the embryo within the vesicle and comparing values with published tables.[11] The latter group of mares had fetuses from 28 to 45 days of gestation on day 1 of quarantine. Physical examinations were conducted once daily while mares were in quarantine. Pregnancy was re-evaluated on the day before the trial began, and 15 of the 18 mares were randomly allocated into 1 of 3 groups. Mares were housed in box stalls beginning 12 days before the trial and through the 21-day trial. They were not exposed to grass during that time. Each mare was hand walked twice daily. All horses were fed commercial grass hay grown in Nevada. Water was available ad libitum in buckets. The project was approved by an animal use committee that followed USDA guidelines published on the USDA Web site.

Procedures and sample collection—Day 0 was defined as the first day the mares were gavaged, and day –1 was defined as the previous day. Physical examinations were performed once daily during the 12-day quarantine period and 3 times daily (at 8 AM, 4 PM, and 11 PM) during the 21-day trial. Blood was obtained at 8 AM each morning for CBCs from day –1 through day 21 of the trial. Examinations included evaluation of rectal temperature, heart rate, respiratory rate, intestinal activity, and digital pulses. In addition, the perineum of each mare was examined for vaginal discharge and the presence of fetal membranes.

Transrectal ultrasonography was performed daily between 5 and 7 PM from day –1 through day 21. The reproductive tract of each mare was examined by 1 of 2 individuals with a portable ultrasound machine with a 5.0-mHz probe.[a] No mares were sedated. All examinations were recorded on videotape and stored for future reference. Neither individual knew the grouping of mares or treatments. The rectum was emptied and the uterus was palpated to confirm pregnancy. The ultrasound probe was inserted into the rectum, and fetus and fetal fluids were scanned. The allantoic fluid was evaluated first. Amniotic fluid was not consistently observed. Allantoic and amniotic fluids were graded, in real time, for degree of echogenicity. Echogenicity of the fetal fluids was graded from 0 to 5 at the time of examination as 0 = anechoic fluid without any echogenic material; 1 = a few hyperechoic particles in the fluid (**Figure 1**); 2 = a moderate number of hyperechoic particles in the fluid; 3 = > 10% to < 50% of the fluid contained hyperechoic particles (**Figure 2**); 4 = some anechoic areas but ≥ 50% of the fluid contained hyperechoic particles (**Figure 3**); and 5 = no anechoic areas seen within the fetal fluid. The fetus was evaluated for movement and heart rate. If the fetus was dead at ultrasonographic examination, it was removed manually. Swab specimens for aerobic bacterial culture were obtained from the body of the aborted fetuses and sent to the microbiology laboratory at Rood and Riddle Equine Hospital. Levine eosin methylene blue agar and thioglycollate broth were used. Eastern tent caterpillar larvae, excreta, and nests were collected from ornamental crabapple (*Malus* hybrid) and wild black cherry (*Prunus serotina*) trees in central Kentucky in late April and early May, when most larvae were in late instar stages (weight, 0.7 to 1.3 g/larva). Larvae were held in 16 × 28 × 40-cm plastic crisper boxes. Air circulation was maintained through wire-screened holes (8 cm in diameter) on each end of the box. The ETC larvae were maintained without food at 5°C until use (2 to 4 weeks). Exceta was separated from larvae and nest material by use of a 0.5-cm sieve. Excreta was stored at –20° or –80°C. Excreta specimens stored at –20° and –80°C were mixed in equal quantities before administration to the mares. Excreta was stored at both temperatures because there was no information on optimal storage conditions.

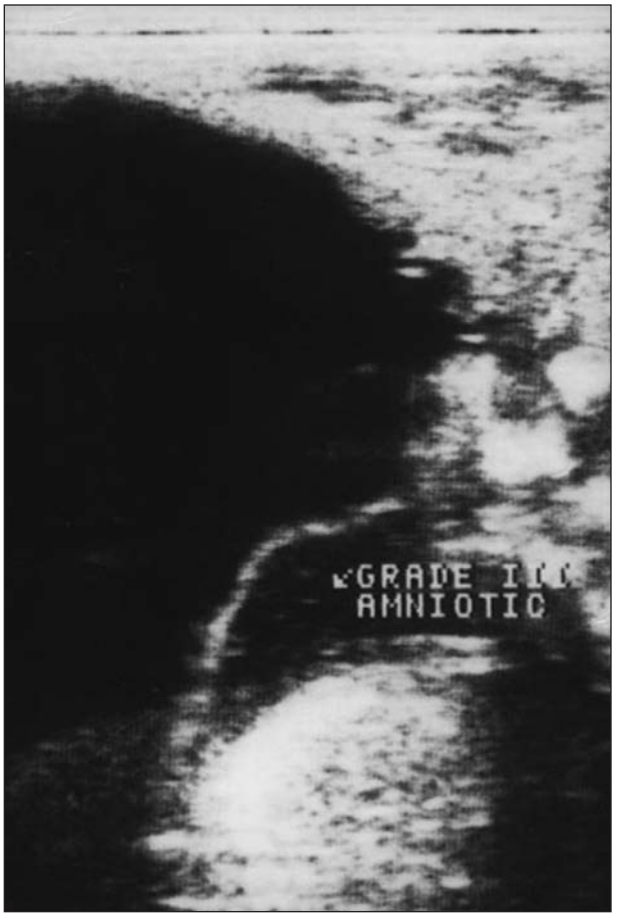

Figure 1—Ultrasonographic image of allantoic fluids in a pregnant mare on day 72 of gestation. Allantoic fluid score is 1.

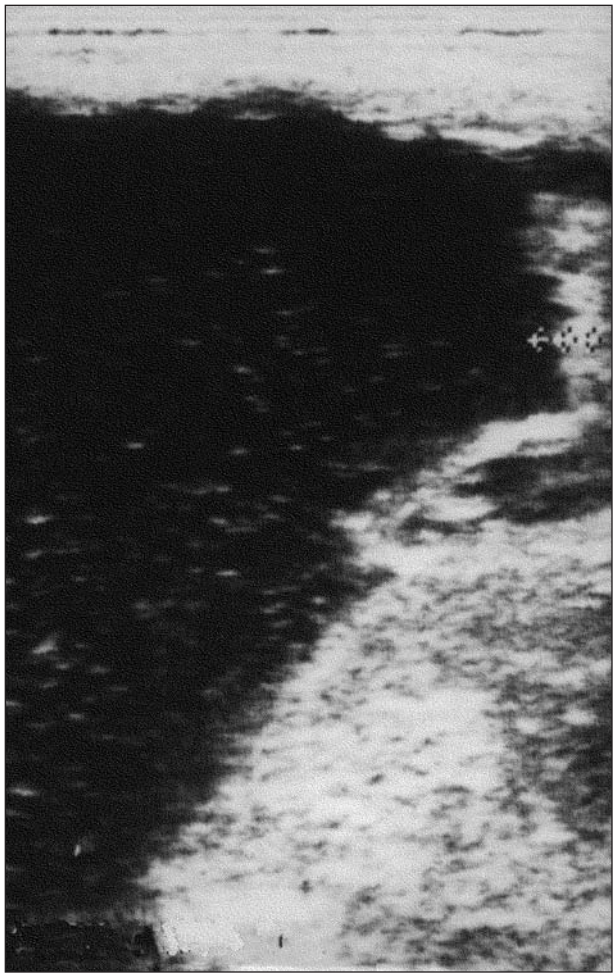

Figure 2—Ultrasonographic image of allantoic fluids in a pregnant mare on day 71 of gestation. Allantoic fluid score is 3.

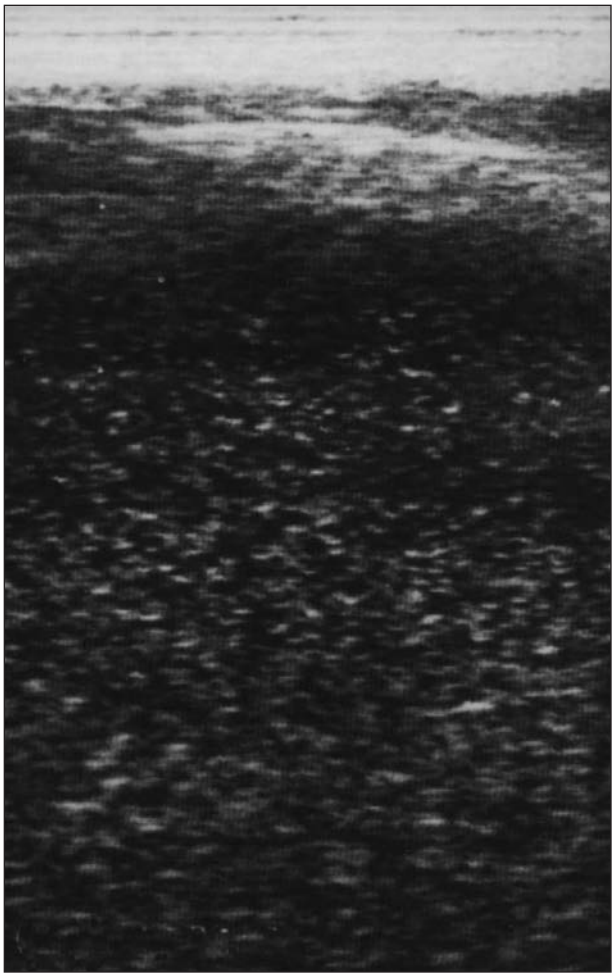

Figure 3—Ultrasonographic image of allantoic fluids in a pregnant mare on day 58 of gestation. Allantoic fluid score is 4. A dead fetus was identified by ultrasonography 48 hours later.

Treatments—Treatments were administered via nasogastric tube daily for 10 days. A separate tube, bucket, and funnel were used for each group of mares. Mares in group 1 received 2.5 g of excreta (equal amounts of excreta stored at –20° and –80°C). Two and one half grams of excreta is approximately the amount of excreta that these insects will produce during a 24-hour period. Thawed excreta was diluted with 50 mL of water and poured down a nasogastric tube through a funnel. The funnel and tube were flushed with 500 mL of water to ensure complete dosing. Mares in group 2 received 50 g of refrigerated, macerated, starved ETCs suspended in 500 mL of water. The funnel and tube were flushed as for treatment 1. Fifty grams of ETC larvae is approximately equivalent to 75 to 100 fifth and sixth instar larvae. Mares in group 3 received 500 mL of water daily via nasogastric tube.

Statistical analyses—Equality of the proportion of mares that aborted in all 3 groups was tested via the Fisher exact test. Parameters from the CBC were initially analyzed by use of repeated measures ANOVA.[12] Treatment (control, excreta, or ETCs) was the explanatory variable. The change in the value from that recorded on day 0 (first day of gavage) for each response variable was analyzed via repeated measures ANOVA because each mare provided data for up to 20 days. Response variables included hemoglobin concentration, PCV, total protein concentration, RBC concentration, and WBC concentration, and evaluations of percentages of neutrophils, immature neutrophils, lymphocytes, monocytes, and eosinophils. Response variables that were significantly different from those of control mares were further analyzed by evaluating differences in the treatment means within each day using 1-way ANOVA.[12]

Results

Four of 5 group 2 mares aborted on trial days 8 (2 mares), 10, and 13 (49, 64, 70, and 96 days of gestation), whereas no mares in groups 1 or 3 aborted (P = 0.01). Three of these fetuses were removed vaginally, and 1 was recovered from the mare's stall. Alpha streptococci were recovered from 1 fetus, and *Serratia marcescens* was recovered from the other 3 fetuses. Results of mare ophthalmologic and cardiac examinations were normal. Physical examination findings were normal in 3 of 5 mares in group 1, in 3 of 5 mares in group 2, and in all control mares. Two mares in group 1 had fevers on days 4 and 9. One of the 2 mares subsequently developed a submandibular swelling and draining abscess. The abscess yielded *Streptococcus equi* subsp *equi*. Isolation procedures used for horses with infectious disease were instituted with the 2 mares. Two mares in group 2 that aborted had abnormal clinical signs approximately 40 hours before a dead fetus was detected via ultrasonography (at the 7 PM

examination). One mare had a fever of 39.78°C (103.6°F) that resolved without treatment by 8 AM the next morning. The second mare had signs of abdominal pain intermittently at night. The mare was treated with 500 mg of flunixin meglumin administered IV, and the signs of pain resolved. Five days after abortion (study day 13), the latter mare had fever ranging from 39.72° to 40.67°C (103.5° to 105.2°F). On day 17 of the trial, a nasal swab specimen submitted for culture of *S equi* yielded negative results. On day 21 of the study, a second swab specimen was submitted for polymerase chain reaction assay and yielded positive results for *S equi*. Mean daily WBC count remained within reference range for the 3 groups. Mean daily WBC count decreased in group 1 mares during the study and was significantly ($P = 0.04$) lower than the mean WBC count of mares in groups 2 and 3. Mean daily WBC count decreased for group 1 (–1,424 cells/mL), increased for group 2 (+1,407 cell/mL), and was fairly constant for group 3 (+289 cells/mL).

Allantoic fluid scores in mares that did not abort ranged from 0 to 2 in mares with fetuses of ≤ 85 days of gestation and from 1 to 3 in mares with fetuses of > 85 days of gestation. Allantoic fluid scores in mares that aborted were similar to those that did not abort until the day before or the day of fetal death, at which time scores were either 4 or 5 in the 4 aborting mares (Figure 3). . Amnionic fluid scores ranged from 0 to 2 throughout the study in all mares except for 1 ultrasonographic recording in a mare that aborted. Amnionic fluid was scored 4 in the aforementioned mare on the day that fetal death was detected.

Discussion

The goals of this study were to determine whether gavage of mares with starved ETCs or their excreta caused EFL and whether ultrasonographic, bacteriologic, and physical findings were similar to those described during the MRLS outbreak in 2001. In addition, blood was obtained to identify changes in CBC parameters. Four of 5 mares gavaged with starved ETCs aborted and had ultrasonographic changes consistent with those observed in affected mares during the spring of 2001 and 2002.[13] Allantoic fluid became hyperechoic either the day before or concurrently with identification of a dead fetus. Changes in the fetal fluid scores as visualized on ultrasonography in experimental mares that aborted were rapid and dramatic. It is not known how rapidly fetal fluids changed in mares affected with MRLS because the reproductive tracts of clinically affected mares were not scanned daily. Normally, allantoic and amnionic fluids in mares with fetuses from 40 to 85 days of gestation are anechoic. After 85 days of gestation, fetal fluids become increasingly more hyperechoic[14] with allantoic fluid scores of 2 and 3 being typical. This pattern was observed in the experimental mares that did not abort. Mares that aborted had allantoic fluid scores of 4 and 5. In our study, dead fetuses were visualized either concurrently or after allantoic fluids became hyperechoic and not before changes in fluid clarity were detected.

We hypothesized that the hyperechoic particles observed ultrasonographically in allantoic fluids of mares with fetuses of > 85 days of gestation that did not abort and in experimental mares that aborted were sloughed epithelial cells from the allantoic membrane. At day 60 of gestation, allantoic epithelium consists of squamous epithelial cells, and by day 80, allantoic epithelial cells take on a more cuboidal form, suggesting increased ectodermal activity. Allantoic fluid volume increases from days 40 to 80 of gestation, and subsequently decreases after day 80.[15,16] Decreased volume of allantoic fluid would concentrate nutrients and epithelial cells floating within the allantoic sac. The high allantoic fluid scores in the mares that aborted from MRLS may have resulted from a toxin or infectious agent that caused increased loss of epithelial cells lining the allantois, decreased allantoic fluid volume, or both. It might also have been a consequence of damage to allantoic blood vessels.

Conception rates in mares and pregnancy rates in mares with fetuses that were < 35 days of gestation are not affected by MRLS. Mares with fetuses from 35 to 120 days of gestation may be more susceptible to a toxic or infectious agent because the yolk-sac placenta is gradually being taken over by the highly vascularized allantois and the placenta is attaching to the endometrium during this time.[15,16] Disruption of the vascularization of the allantois could result in fetal damage and death. During the period of placental attachment (days 40 to 120), the fetus is growing at a rapid rate.[17,18] Inability of the placenta to attach properly to the endometrium could result in decreased nutritional support to the fetus and eventual fetal death.

Alpha-hemolytic streptococci were the most common isolates recovered from affected mares in 2001 and 2002.[19] In our study, a *Streptococcus* sp was isolated from only 1 of the 4 aborted fetuses. *Serratia marcescens* was recovered from the other 3 fetuses. *Serratia* spp were rarely (approx 1%) recovered from mares affected with MRLS[19]; however, *Serratia* spp were also isolated from aborted fetuses in an experimental model that used mares in late gestation.[20] Mares in that study were gavaged with caterpillars collected from Michigan. Both species of bacteria may be derived from insects, soil, or the digestive tract of horses.

Alpha-hemolytic *Streptococcus* spp and *Serratia* spp are uncommonly recovered from the uterus or aborted fetuses. It is not known whether these organisms are the cause of abortion in MRLS or if their recovery is a secondary finding (ie, opportunistic infection). Although alpha-hemolytic *Streptococcus* spp are not considered uterine pathogens, they have been recovered from the uterus, vagina, vestibule, and clitoral fossa of reproductively normal mares. In a study in which swab specimens for bacterial culture were obtained from the uterus, vagina, vestibule, and clitoral fossa of 48 mares that had normal reproductive tracts, no history of reproductive problems, and no inflammation on evaluation of endometrial biopsy specimens, alpha-hemolytic *Streptococci* spp were recovered from 44 of 368 (12%) specimens.[21] Only 2 uterine swab specimens obtained from the 48 mares in that study yielded more than 10 colonies; 1 of the specimens yielded only an alpha-hemolytic *Streptococcus* sp.

A number of theories has been put forth regarding the reason these organisms are recovered from fetuses of mares with MRLS. One theory is that alpha-hemolytic streptococci are opportunistic bacteria found in the vagina and they gain entry into the uterus through a dilated

cervix. The cervix may dilate secondary to release of proinflammatory cytokines, PGE_2, and $PGF_{2\alpha}$. It is proposed that cytokines may be induced directly by the toxic component of ETCs or as a consequence of proliferation of alpha-hemolytic streptococci in the caudal portion of the reproductive tract of mares. A second theory is that ETCs harbor the bacteria within their setae (hairs). The bacteria may gain entry into the systemic circulation of the mare by penetrating a mucous membrane, such as that of the oral or nasal cavity, the vagina, or gastrointestinal tract. The bacteria may have a predilection for the uterus because the placenta is undergoing rapid vascularization and growth. A third theory is that the caterpillar irritates the gastrointestinal mucosa, permitting entry of bacteria from within the lumen of the mare's gastrointestinal tract into the systemic circulation.

On the basis of results of the previous field study, it was somewhat surprising that mares administered excreta did not abort or have changes in allantoic or amnionic fluid scores. In an earlier study, 7 of 9 mares exposed to pastures that were contaminated with ETC excreta aborted.[10] Abortions occurred in the pastures after application of relatively small amounts of excreta (< 1 kg) to the pasture.[10] As mentioned, it is possible that escaped ETC larvae caused these losses. Alternatively, excreta used in our study was from older insect larvae, more rigorously separated from other nest materials (through a sieve), stored at –5° and –80°C, thawed, and mixed equally before it was administered. The dose administered may have been too small, or the toxic compound or infectious agent within the excreta may have been damaged or destroyed by the freeze-thaw cycle.

Although administration of excreta did not induce abortion, it was associated with a decrease in the daily mean WBC count, fevers in 2 mares, and possibly immunosuppression (1 mare developed a submandibular abscess). The decrease in WBC count was likely not of clinical importance because the values remained within the laboratory's reference range and the mares did not abort. Furthermore, mares fed starved ETCs did abort and did not have decreased daily mean WBC count. Daily mean WBC count may have decreased over time in mares fed excreta because those mares may have had alterations in the gastrointestinal flora, possibly associated with dose. It did not appear that *S equi* in the 2 mares contributed to the decreased WBC count because the mean WBC count in the 2 mares was consistently greater than the mean for their group and the WBC count continued to increase during the trial. We can only speculate whether the submandibular abscess in the mare in group 1 and the eventual detection of *S equi* by use of the polymerase chain reaction assay in the mare 8 days after abortion was associated with the feeding of excreta or ETCs.

This report confirms that ETCs are a causative agent of EFLs associated with MRLS. Ultrasonographic changes observed in fetal fluids were typical of MRLS; however, bacteriologic findings were not typical of culture results from mares with MRLS. Associations between bacteria and MRLS or ETCs remain to be elucidated, as does the possibility of a chemical toxin or biological agent resulting in fetal or placental damage. However, the knowledge that the ingestion of ETCs is somehow causative for MRLS can assist in better management and prevent future losses to the horse industry.

[a]Aloka SSD 900, Aloka Co Ltd, TD Wallingford, Conn.

References

1. Williams NM. Mare reproductive loss syndrome: pathologic findings. *Equine Disease Quarterly* 2002;10(4):4–5.
2. Smith MS. Mare reproductive loss syndrome: research status report and executive summary. Available at: www.uky.edu/Agriculture/VetScience/mrls/summary914.htm. Accessed Sept 14, 2001.
3. Morehead JP, Blanchard TL, Thompson JA, et al. Evaluation of early fetal losses on four equine farms in central Kentucky: 73 cases (2001). *J Am Vet Med Assoc* 2002;220:1828–1830.
4. Bain FT, Williams NM. Clinical overview of fetal loss and neonatal problems in the Kentucky outbreak of 2001, in *Proceedings*. 20th Ann Forum Am Coll of Vet Inter Med 2002;135.
5. Reimer J. Pericarditis outbreak: management and prognosis, in *Proceedings*. 20th Ann Forum Am Coll Vet Inter Med 2002;133–134.
6. Latimer C. Endophthalmitis: a syndrome associated with mare reproductive loss syndrome?, in *Proceedings*. 1st Workshop Mare Reprod Loss Syndrome 2002;17–20.
7. Paulick R. MRLS Economic impact: $336 million. Available at: www.bloodhorse.com. Accessed Oct 15, 2001.
8. Dwyer RM, Garber LP, Traub-Dargatz JL, et al. A case-control study of factors associated with excessive proportions of early fetal losses associated with mare reproductive loss syndrome in central Kentucky during 2001. *J Am Vet Med Assoc* 2003;222:613–619.
9. Cohen ND, Donahue JG, Carey VJ, et al. Case-control study of early-term abortions (early fetal losses) associated with mare reproductive loss syndrome in central Kentucky. *J Am Vet Med Assoc* 2003;222:210–217.
10. Webb BA, Barney WE, Dahlman DL, et al. Induction of mare reproductive loss syndrome by direct exposure of susceptible mares to eastern tent caterpillar larvae and frass, in *Proceedings*. 1st Workshop Mare Reproductive Loss Syndrome 2002;78–79.
11. Ginther OJ. The single embryo. In: *Ultrasonic imaging and reproductive events in the mare*. Cross Plains, Wisc: Equiservices, 1986;196–228.
12. SAS Institute, Inc. *JMP statistics and graphics guide*. Cary, NC: SAS Institute, Inc, 2001.
13. Riddle WT. Clinical observations associated with early fetal loss in mare reproductive loss syndrome during the 2001 and 2002 breeding seasons, in *Proceedings*. 1st Workshop Mare Reprod Loss Syndrome 2002;12–14.
14. Vince KJ, Riddle WT, LeBlanc MM, et al. Ultrasonographic appearance of fetal fluids between 55 and 176 days of gestation in the mare: effect of mare reproductive loss syndrome, in *Proceedings*. 48th Ann Meet Am Assoc Equine Pract 2002;350–352.
15. Ginther OJ. Embryology and placentation. In: *Reproductive biology of the mare and applied aspects*. 2nd ed. Cross Plains, Wisc: Equiservices, 1992;380–389.
16. Bazer FW. Allantoic fluid: regulation of volume and composition. In: Brace RA, ed. *Reproductive and perinatal medicine*. Vol XI. Ithaca, NY: Perinatology Press, 1989;135–155.
17. Samuel CA, Allen WR, Steven DH. Studies on the equine placenta. I. Development of the microcotyledons. *J Reprod Fertil* 1974;41:441–445.
18. Steven DH, Samuel CA. Anatomy of the placental barrier in the mare. *J Reprod Fertil Suppl* 1975;S23:579–582.
19. Donahue J, Sells S, Bolin D, et al. Bacteria associated with mare reproductive loss syndrome: late fetal losses, in *Proceedings*. 1st Workshop Mare Reprod Loss Syndrome 2002;27–29.
20. Sebastian M, Williams D, Harrison L, et al. Experimentally induced mare reproductive loss syndrome: late fetal losses with eastern tent caterpillars, in *Proceedings*. 1st Workshop Mare Reprod Loss Syndrome 2002;80–81.
21. Hinrichs K, Cummings MR, Sertich PL, et al. Clinical significance of aerobic bacterial flora of the uterus, vagina, vestibule, and clitoral fossa of clinically normal mares. *J Am Vet Med Assoc* 1988;193:72–75.