Update on Mare Reproductive Loss Syndrome

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In the spring of 2001 and 2002 Central Kentucky experienced large numbers of early and late term fetal losses in mares of multiple breeds. In addition to the reproductive losses, a number of pericarditis and endophthalmitis cases were identified in Central Kentucky horses during the same time period. Together these reproductive and non-reproductive cases have been termed Mare Reproductive Loss Syndrome. Other areas of Kentucky and neighboring states also reported smaller numbers of cases consistent with MRLS.

The economic impact to Kentucky from the 2001 losses was estimated to be nearly $336 million according to a study conducted by the University of Louisville. In 2001 there were approximately 550 late term losses, between 2000 and 3000 early fetal losses, approximately 30 endophthalmitis cases, and between 50 and 60 pericarditis cases. In 2002 fewer cases were seen, with 165 late term losses, approximately 500 early fetal losses, 6 endophthalmitis cases and 9 pericarditis cases being reported. The economic impact from MRLS will be felt for years following the actual cases. The 2002 Keeneland November Breeding Stock Sale was the smallest since 1993, with 864 foals entered compared to 1063 in 2001 and 1379 in 2000. The 2003 Keeneland July Select Yearling Sale was cancelled with a primary reason being fewer yearlings available as a result of MRLS losses.

Early Fetal Losses[a]

Mare Reproductive Loss Syndrome was first seen when pregnant mares between 60 and 70 days gestation were being examined by ultrasound to determine the sex of their fetuses. Beginning on April 26, 2001 some of these mares were found to have dead fetuses surrounded by allantoic and amniotic fluids containing hyperechoic material. This was the beginning of an unprecedented number of early abortions in Central Kentucky. A report released by the governor's office estimated the number of early fetal losses caused by MRLS in 2001 to be 2,998.

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 early fetal loss were seen in Central Kentucky. Estimates were 256 in 1980 and 162 in 1981. 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 early fetal loss 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) one to three days before early fetal loss occurs.

[a] A portion of this section was presented at the First Workshop on Mare Reproductive Loss Syndrome on August 27 and 28, 2002 at the Maxwell H. Gluck Equine Research Center at the University of Kentucky, Lexington, Kentucky.

Often with MRLS losses rectal palpation will indicate the mare is pregnant. The uterus may have normal to slightly less fluid distention 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 in Central Kentucky 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 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 breedings 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.

Gestational Ages Represented

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

Table 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 which I service. In 2002 I had eighteen losses which 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 which aborted in my practice was as follows:

Barren	19	(34%)
Maiden	20	(36%)
Foaling	17	(30%)

Thus barren, maiden and foaling mares were roughly equally represented.

In 2002 the status of the mares which aborted was as follows:

Barren/Aborted	5	(28%)
Maiden	10	(56%)
Foaling	3	(16%)

Maidens represent 56% of losses in 2002 which is likely the result of heavy eastern tent caterpillar loads in maiden fields on one farm.

In 2001 serum progesterone levels measured by Elisa from 10 mares taken when early fetal loss was found averaged greater than 4 ng/ml. In 2002 serum progesterone levels

from 6 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.

In 2001 and 2002 uterine cultures were taken on all mares in my practice following abortion. Results included alpha Streptococcus, beta Streptococcus, Eschericia coli, Enterobacter cloacae and no growth.

Uterine cytologies taken within 7 days of abortion from 5 mares in 2001 showed moderate to severe inflammation. This inflammation is consistent with a 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 which 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 who were less than 80 days of gestation with live fetuses and echogenic fluids. Of these 29 mares, eight or 27.5% aborted within 30 days. This can be compared to May 2002 records which show 69 mares less than 80 days with live fetuses and echogenic fluids. Only 5 of these mares, or 7%, 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 eighty to ninety 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[1].

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 seventy four 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. In 2001 by mid June in Central Kentucky, 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. In August 2002, there was an increase in the percentage of mares with echogenicity before 80 days. No early fetal loss was associated with this increased echogenicity.

In 2001, when MRLS was first identified, 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 term abortions showed some signs consistent with fescue toxicity (premature placental separation and thickened placentas).
3. Mycotoxin binders – These were used in cases where there was a fungal pathogen.
4. Flunixin meglumine – This was used for its anti-inflammatory and anti-endotoxin functions.
5. Pentoxifylline – This was recommended by some practitioners for its reagenic properties.

In hindsight these treatments did not appear to be of any help. Regu-Mate or progesterone were not recommended because progesterone levels in aborted mares had not indicated a progesterone deficiency.

When considering the cause for MRLS, my clinical impression strongly supports the eastern tent caterpillar as the cause. In 2002, efforts at prevention were focused on limiting pasture turn-out and reducing or eliminating exposure to the eastern tent caterpillar. 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 which 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 two to six 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.

Late Term Losses

In addition to the early fetal losses a smaller, but significant number of mares (approximately 550 in 2001 and 165 in 2002 [2] were experiencing late term fetal loss during the same time period in late April and May. These losses occurred in the last trimester, with most within 30 days of the expected foaling date. The majority of these mares exhibited no signs of impending foaling or abortion. Labor was reported to be very intense and was often characterized as explosive. Of the fetuses submitted to the Livestock Diagnostic Disease Center, 32% were from deliveries with premature separation of the placenta and 11% reported dystocia[3].

The resulting foals from these late term MRLS cases were either dead at birth or extremely compromised. Clinical signs were consistent with asphyxia and the foals most often required resuscitation[4]. Surviving foals were referred to intensive care facilities. Upon admission the foals were found to be dehydrated, hypothermic, and tachycardic, with irregular respiration. Leukopenia, hypoglycemia, and acidosis were consistently noted. Bilateral hyphema was seen in a significant number of foals. Therapy included resuscitation, supportive care, and antibiotics. There was a poor prognosis for survival.

Significant pathology in the late term MRLS fetuses included inflammation of the amnionic segment of the umbilical cord, inflammation of the amnion, pneumonia, fetal bacteremia, and occasionally placentitis[5]. The most remarkable finding was the appearance of the amnionic umbilical cord, which in many cases was edematous and grayish-yellow in color, with a roughened and hemorrhagic surface. Because there was no single laboratory test or necropsy finding that confirmed a diagnosis of MRLS in the late term foal, diagnosis was made based on a combination of factors, which included history, time of year, bacteriology, and pathology[5].

Pericarditis

Pericarditis was one of two non-reproductive syndromes associated with MRLS. In 2001 and 2002 outbreaks of fibrinous, effusive pericarditis were seen concurrent with the occurrence of early and late term fetal loss[6]. These cases occurred in all ages, genders and multiple breeds. Epidemiologic studies which examined the cases of pericarditis and MRLS found that the two diseases were highly associated with each other[7]. However, mares with pericarditis were not at increased risk to experience fetal loss[8].

Endophthalmitis

In addition to pericarditis, a second non-reproductive syndrome, endophthalmitis, was associated with MRLS. This condition was characterized by inflammatory debris in both the anterior and posterior segments and was seen in horses of all ages and genders and multiple breeds[9]. It is interesting to note that all cases were unilateral and were unresponsive to aggressive therapy. Without exception, all horses became blind in the affected eye.

MRLS Research

In the summer and autumn of 2001 numerous theories were proposed for the cause of MRLS. Some theories centered on the ingestion of toxic substances in the grass in pastures while others implicated the Eastern Tent Caterpillar. Theories associated with the grasses included: electrolyte imbalances in grasses induced from repeated freeze-thaws in the spring, nitrate-nitrite toxicity, ingestion of poison Hemlock , white clover, tall fescue alkaloids or grasses that contained myotoxins. Ingestion or inhalation of the Eastern Tent Caterpillar was thought to be associated with the absorption of either a biological (bacteria, virus or parasite) or chemical toxin. In the winter of 2001, an epidemiologic survey conducted by the University of Kentucky identified four factors associated with increased MRLS: breeding date in February 2001; moderate to high concentration of eastern tent caterpillars (Malacosoma americanum) in mare areas; presence of wild cherry trees around pastures; and having more than 50 mares on the farm [10] . Two factors were associated with a low incidence or no incidence of MRLS: absence of caterpillars; and feeding hay to mares in pasture. A subsequent case control study of early term abortions found 5 factors to increase the risk of EFL: 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 heavier burden of caterpillars in pastures during 2001; prior abortion during the previous 5 years; elk or deer being seen at the premises during the preceding 12 months [11].

Based on the above-mentioned risk factor of caterpillar exposure and MRLS a trial feeding caterpillars and their frass to pregnant mares was undertaken in the spring of 2002. Pregnant mares housed on pasture were exposed to either ETC with frass or frass alone. Ten mares housed under identical conditions were maintained as controls. Treatments were sprinkled on pasture to which mares were exposed for 6 hours per day. In the ETC/frass treatment group 7/10 mares aborted. In the frass group 7/10 mares aborted. In the control group 3/10 mares aborted [12].

A second study was designed to complement the previous study by removing the mares from exposure to grass and to more strictly control the treatments of ETC and their frass [13]. Fifteen mares between 40-80 days of gestation were housed in stalls for a quarantine period of 12 days and then gavaged with either 2.5 g of frass, 50 g of starved Eastern Tent caterpillars, or 500 mls of water for 10 days (five mares in each group). Physical examinations were conducted every 8 hours and the reproductive tract was evaluated by ultrasonography once daily. Four of the 5 mares gavaged with ETC aborted on trial days 8, 10 and 13. No mares fed frass or gavaged with water aborted. Ultrasonographic findings were similar to that seen in clinical cases. The allantoic fluid became hyperechoic either before the fetus died or was associated with fetal death.

Additional feeding trials were conducted in 2002 with the ETC to determine if it was associated with late term losses, to possibly identify the toxic principle or the portion

of the caterpillar associated with the syndrome. In the first study mares in late gestation were gavaged with either 50 g of Eastern Tent Caterpillars or water daily [14]. Six of six mares fed caterpillars aborted with the first abortion occurring 68 hours after the first treatment. None of the control mares aborted.

As there were questions on what portion of the caterpillar contained the toxic compounds, a small pilot study was conducted. Mares were either fed the integument of the caterpillar or the gastrointestinal contents of the caterpillar. Two of 5 mares fed the integument aborted while none of the mares fed the gastrointestinal contents aborted. Other studies were conducted in an attempt to identify the toxic principle or to determine if freezing of the caterpillar diminished the toxicity. In the first study caterpillars were autoclaved to remove biological toxins and fed to mares. No mares fed autoclaved caterpillars aborted (n=5). Unfortunately, it was discovered that autoclaving destroys many chemical toxins so the study was not able to clearly delineate if the toxic substance was a chemical or biological compound. In the latter study mares were fed frozen caterpillars. Three of five mares aborted so it appears that freezing of the whole caterpillar does not destroy the toxin.

Conclusions that can be made from the studies conducted in 2002 are that ingestion of the ETC is associated with both early and late term fetal losses. Ultrasonographic changes of the reproductive tract are similar to those observed in clinical cases. The toxin appears to be in the integument. Freezing does not destroy the toxin whereas autoclaving does destroy the toxin.

Work to be conducted in the spring and summer of 2003 centers on identifying if the toxin is a biological or chemical agent. Eastern Tent Caterpillars will be irradiated to remove biological agents and fed to mares in late gestation. If these mares abort, data will strongly implicate either a bacteria, virus or parasite as the cause. If mares do not abort, then it is most likely that the caterpillar contains a chemical agent that disrupts the pregnancy. Further studies will then need to be performed to isolate the agent.

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