In the spring of 2001, central Kentucky was devastated by what came to be known as Mare Reproductive Loss Syndrome (MRLS). Pregnant mares aborted early- and late-term or gave birth to stillborn and weakened foals by the hundreds. There were also cases of unilateral uveitis and pericarditis at the same time as the abortions. Horses of many different breeds were affected, however the thoroughbred breeding farms were hit the hardest. A list of possible culprits was quickly assembled, and the daunting task of eliminating them one by one began. Popular theories of cyanide poisoning and mycotoxins were eventually set aside due to lack of evidence, and the focus was put on the eastern tent caterpillar. There was an obvious direct relationship between the eastern tent caterpillar and the occurrence of MRLS. However, how the caterpillar was causing the losses remains to be determined. Potential mechanisms are discussed along with potential means of prevention.

Introduction

In the spring of 2001, central Kentucky was devastated by what came to be known as Mare Reproductive Loss Syndrome (MRLS). The syndrome began to wreak havoc at the end of April, and resulted in early- as well as late-term abortions, sick and weakened foals, stillbirths, and occasional pericarditis and unilateral uveitis. The incidence of the syndrome peaked during the first week in May, and by the end of June, it had practically ceased. In those few, agonizing weeks, the syndrome claimed approximately 500 late-term fetuses and approximately 2,000 early-term fetuses. Several breeds were involved with these losses, however, the impact was the greatest on the thoroughbred breeding farms. Approximately 30.5% of the foals expected in 2002 were lost, resulting in losses estimated at $300.5 million. The purpose of this paper is to outline the progression of mare reproductive loss syndrome, and to discuss the seemingly endless search to find its cause.

The Epidemic of 2001

April 26, 2001 marked the start of a disastrous new syndrome that began to tear through central Kentucky. On April 26, Tom Riddle, DVM, visited a thoroughbred breeding farm to fetal-sex six mares. This procedure is quite common, and involves ultrasound to determine if the foals to be born are fillies or colts. Although the procedure was familiar to Dr. Riddle, he was surprised to find that two of the six mares were carrying dead fetuses. Being concerned, he spoke with the farm's veterinarian, and together they determined that the two losses were merely a case of bad luck.

On April 27, Dr. Riddle visited another thoroughbred breeding farm in a different county in Kentucky, and found two more dead fetuses while fetal-sexing another six mares. At this point, Dr. Riddle, the farm veterinarian, and the farm
manager decided that the two cases of fetal death may be related. In just sixteen hours since the first report of dead fetuses on April 26, the reports of fetal deaths began to come in from many other farms. On April 28, two days after the initial finding of dead fetuses, it was concluded that Kentucky was dealing with a serious problem that was spreading epidemically. At the same time, a major equine practice in the Lexington area reported a dramatic increase in the number of sick foals being brought to the clinic. Along with the sick foals, farms brought the news of an increase in stillbirths. The sick foals were presenting with trembling, low-grade fever, and a loss of appetite, all with very few of their dams being sick. The foals were extremely ill and appeared to be septic. They were severely dehydrated, with low blood sugar, and very low white blood cell counts. The dams did not seem to be affected until they aborted, both late-term and early-term abortions were seen. The aborted fetuses showed no signs of necrosis, meaning that they were viable for some time before being aborted.

May 1, 2001 was the start of the worst weeks. The number of dead fetuses and sick foals had been spiraling upward, seemingly out of control. At this point, it was also realized that broodmares and foals were not the only horses affected. The syndrome had also been affecting horses of many different breeds, ages, and both sexes. At this time in May, it was realized that there was also a dramatic increase in cases of pericarditis (inflammation of the sac around the heart) and uveitis (inflammation of the interior of the eye). In the Bluegrass, veterinarians reported seeing around one or two horses with pericarditis in a year. In one week, they had seen twenty. The pericarditis cases were also unusual. Pericarditis is commonly a secondary problem following a bacterial or viral infection. However, no viruses or bacteria could be found in these cases. Fluid is normally removed from the pericardial sac when treating pericarditis. There is normally approximately one liter to twenty-one liters drained. In these cases, there was an extreme build up of fluid. One case was documented to have had around five gallons of fluid drained from the pericardial sac. Interestingly, none of the horses with pericarditis also presented with uveitis. By May 8, the number of horses and foals being affected by the syndrome was still rising. As of noon on May 8, there were 318 aborted fetuses and dead foals reported. Since April 26, the deaths were occurring at a rate of about thirty-one per day.

At this point in time, there were preliminary results of necropsy examinations, and the beginning of a long list of possible culprits. Necropsy examinations showed lesions in both the fetus and the placenta. Hyphema (blood in the eyes) was sometimes present. The placentas showed edema and amniotic inflammation, and hemorrhages were often present on the lungs and heart. There were two areas of great interest in the examinations. The first was dealing with the respiratory changes. The lungs were dark and firm, suggesting pneumonia. They also contained amniotic fluid, that had apparently been sucked in while in utero. These findings in the lungs suggested that the foals were struggling and under respiratory distress while in utero. The other area of interest was the umbilical cord. A large percentage of the examined cases presented a roughening of the surface and enlargement of the umbilical cord. There was also discoloration of this area. It was a dull grayish-yellow color, with some hemorrhaging. Interestingly, these umbilical changes were limited to the amniotic segment. The allantoic portion appeared to be normal in color and texture. Upon these examinations, preliminary thoughts were being born as to what was causing the deaths.

The very first tests that were done were necropsy examinations, and nutritional tests on the mares that aborted. The fetuses tested negative for nitrates and nitrites. Along with these results, no viral or bacterial agents were isolated from the necropsy examinations. Nutritional imbalances were also ruled out by testing the mares for levels of copper, iron, selenium, and zinc. All samples tested were within the normal range. All samples were also tested for equine herpes virus 1 and 4, equine arteritis virus, adenovirus, and leptospirosis. All samples tested negative. These results shifted the investigation to environmental factors. The first list of culprits included phytoestrogens (plant hormones), estrogenic mycotoxins (estrogen like fungal toxins), and ergot alkaloids (plant fungus derived chemicals). However, one specific mycotoxin was a primary suspect. Zearalenone is a...
mycotoxin produced by fusarium mold, and is known to cause abortions in pigs. There was also another factor that coincided with the zearalenone theory. The Bluegrass region had seen abnormal weather changes in the spring of 2001. There was a period of drought followed by a freeze. These weather patterns are perfect for the acceleration of mold growth. The outlook seemed positive for proving that zearalenone was the culprit for the epidemic.

By May 15, the number of deaths due to the epidemic had slowed. Every few days the state veterinarian's office communicated with state practitioners, and for the first time, the syndrome was given a name. The epidemic was to be known as mare reproductive loss syndrome (MRLS). The given name was broad and vague, a consequence of the overwhelming unknowns.

Pasture conditions were still of primary concern, and samples had been tested from both affected and unaffected farms. Many of the samples tested from affected pastures showed high levels of zearalenone. However, the levels were not high enough, nor consistent enough, and zearalenone was shortly ruled out. Ergot alkaloids and phytoestrogens also failed to be seen in any tests. As June began and ended, the reported cases of MRLS also ended. The Bluegrass region took a breath of relief. The dying had ceased, but almost instantaneously, they began scrambling for an answer to prevent MRLS from happening again.

Who's to blame?

Each possible culprit that had been considered lead investigators and veterinarians to dead ends. What was left that could be causing MRLS? By the end of the MRLS epidemic in 2001, the investigations quickly changed direction and headed straight for the wild black cherry trees and their inhabitants, the eastern tent caterpillars. The hypothesis at this point was that the cyanide from the black cherry trees was poisoning the fetuses. The levels of cyanide however, were not high enough to poison the mares. Were the mares eating the trees or was the cyanide getting to them by means of the eastern tent caterpillar? This was the question on everyone's mind, as the foaling season of 2002 crept closer and closer.

Experiments and investigations began with the focus on cyanide toxicity. Cyanide was found in pastures, in samples from necropsy examinations, and in the eastern tent caterpillars themselves. It is also known that the leaves of the black cherry tree are cyanogenic, and it is possible for them to cause mortality of livestock if eaten directly. The primary inhabitants of the black cherry trees are the eastern tent caterpillars, who eat the cyanogenic leaves and thus could possibly contain some level of cyanide within them.

Despite the widespread excitement over the new direction of interest, the cyanide theory was not airtight, and many people were skeptical. Studies were done that involved assessing levels of cyanide in the eastern tent caterpillar, and the level of cyanide in the leaves of the black cherry trees.

Studies were first done to assess levels of cyanide in the eastern tent caterpillar. The eastern tent caterpillar primarily lives in the black cherry tree and feeds on its leaves. The caterpillars will leave their tree only under two conditions. The first being if their tree becomes defoliated, and they have to look for a new source of food. The other is when they are ready to find a place to pupate. In the first instance, the caterpillars are devoid of food, hence they are forced to go search for more. In this case, the caterpillars do not contain food in their guts, and therefore do not contain cyanide. In the second circumstance however, the caterpillar is fully grown and full of food. In this case, the caterpillar may contain cyanide in the food in the gut. However, it has been proven that as the cyanide passes from the leaves of the black cherry tree, through the caterpillar, the level of cyanide is greatly reduced. Although not completely eliminated, the level of cyanide has proven to be not enough to cause cyanide poisoning in the horses or fetuses plagued by MRLS. Similarly, other studies have also proven that cyanide poisoning does not cause early- or late-term abortions.
Pregnant mares have been given cyanide to the level of toxicity, and no abortions resulted. From here, the cyanide studies shifted to the leaves of the black cherry tree itself. Could the mares be eating the actual leaves and receiving cyanide from them? Studies were done to evaluate the relationship between MRLS and the black cherry trees. However, upon making the leaves readily available to mares, there was no elevation in the incidence of MRLS. At the same time, the poison hemlock was readily available and also did not produce any abortions. The poison hemlock was yet another possible culprit that researchers wanted to assess. As a result, the promising cyanide theory along with the poison hemlock possibility, were put on the pile with all of the other possible culprits that had been eliminated.

MRLS in 2002

As the spring of 2002 approached, the equine industry seemed to collectively hold its breath. Since the epidemic in 2001, veterinarians, researchers, and investigators had been relentlessly waging war on mare reproductive loss syndrome. There were now specific characteristics upon necropsy examination that could be linked with MRLS. Possible causative agents had been analyzed, and a long list of possible culprits had been eliminated. However, the mystery was not yet solved, and the equine industry went into the 2002 foaling season with the best preparation they could. The popular cyanide theory had been virtually disproved, and most now looked to establish a relationship between MRLS and the eastern tent caterpillars. Breeding farms did the best they could to try to limit their mares’ exposure to the eastern tent caterpillar and the black cherry trees. The researchers were also armed and ready to have tests running and experiments in order for the foaling season of 2002.

As April 2002 began, so did the cases of MRLS. The syndrome was striking again, however, losses seemed to be fewer. Between April 28 and May 25, 270 aborted fetuses were submitted for examination and declared to be due to MRLS. This number was significantly smaller than the 495 abortions reported in the same time period in 2001.10,11 As April gave way to May, a select few farms that were designated as sentinel farms were undergoing analysis. Week after week, the levels of mycotoxins, alkaloids, and cyanide were measured. These and all soil characteristics remained in the normal and non threatening ranges.11 However, some of the sentinel farms also began to experience losses that upon examination were consistent with MRLS. If the pasture and soil characteristics were normal, what was causing the abortions? The one factor associated with the most MRLS cases was exposure to eastern tent caterpillars. Furthermore, farms that had problems in 2001 with MRLS and the presence of eastern tent caterpillars, but did not have the caterpillars in 2002, remained completely free of cases consistent with MRLS.10

By May 1, 2002 the first caterpillar field study yielded results. Ten pregnant mares were in a pasture that was seeded with eastern tent caterpillars. Nine mares were in a pasture that contained the frass (excrement) of the eastern tent caterpillars, and ten mares were in a pasture free of caterpillars and their frass. Six of the mares in the field with caterpillars aborted, six mares in the pasture with frass aborted, and three mares in the control pasture aborted.11 Unfortunately, the distance between the pastures as well as the physical barrier had not been successful in keeping the caterpillars in the right pasture and not in the others. As a result, a second study was performed in late May. The control group was placed in a pasture on a hilltop fifty yards away from the other pastures. An insecticide barrier was also applied to control the caterpillars. This trial yielded results that were much more conclusive, and drew the realization of the culprit of MRLS closer. Of eight mares in the caterpillar infested pasture, three aborted. In the pasture treated with the caterpillar frass, none of the mares aborted, and in the control pasture, one mare aborted.11 From this study, it was concluded that the eastern tent caterpillar did indeed play a role in the abortions associated with MRLS, and due to the many variables that need to be considered, their frass could not be completely ruled out.

Finally, a similar, but more controlled study was conducted. For ten...
days, three groups of five pregnant mares were identically managed except for being given three different liquids via a nasogastric tube. One group was given eastern tent caterpillars in solution, another was given frass in solution, and the last group was given only water and used as a control group. Four of the mares given the caterpillar solution aborted, and none of the mares treated with frass or the control group aborted. On June 11, 2002 there was a scientific link between eastern tent caterpillars and MRLS. As the link became known, the final statistics were calculated for the year. Sick foals were seen, but compared to 2001, they were less in numbers and were overall less sick. The number of abortions reported were down by about 200 from the same period in 2001, and only 164 of the 817 cases were classified as MRLS related losses. The equine industry had successfully navigated through the 2002 foaling season with less damage from mare reproductive loss syndrome, and more importantly, emerged with knowledge that could determine the cause of the deadly syndrome.

The Eastern Tent Caterpillar and MRLS in 2003

Researchers, scientists, and veterinarians began looking at the eastern tent caterpillar, and trying to determine its role in MRLS. The first hypotheses were that the caterpillar could be creating an anaphylactic reaction in the gastrointestinal tract of the mares. Another was the possibility that if a horse ingested a caterpillar, the caterpillar could provide a means for a pathogen to invade. This could be accomplished by the setae or the sharp crotchets of the prolegs. These protrusions could possibly irritate the mucous membranes of the gastrointestinal tract and create a pathway for infection.

It had generally been agreed that the eastern tent caterpillar was directly involved in the occurrence of MRLS. However, its actual role in the involvement remained to be determined. Nonetheless, thoroughbred breeders in the Bluegrass region took precautions in limiting their pregnant mares’ exposure to the caterpillars during the foaling season of 2003. Black cherry trees were removed when possible, and trees that could not be removed were sprayed with pesticide to kill the caterpillars. These measures did indeed affect the concentration of eastern tent caterpillars in 2003. The measures taken were deemed successful when the death toll for 2003 was calculated. Approximately 2,000 mares were examined at sixty days of gestation, and only sixteen of them were carrying a nonviable fetus. Of the sixteen abortions, only six were classified as characteristic of MRLS. The number of abortions in 2003 were lower than the number in 2002 and remarkably lower than the number in 2001. In April through June of 2001, greater than 50% of pregnant mares aborted due to MRLS. In the same time period of 2002, less than 10% aborted from MRLS, and in 2003 less than 0.3% of the pregnant mare abortions were suspected abortions from MRLS. There was also evidence that any loss in the 2003 foaling season suspected to be from MRLS was a result of difficulty in completely eliminating exposure to the eastern tent caterpillars.

Limiting pregnant mare exposure to eastern tent caterpillars proved successful in the foaling season of 2003. However, more research needed to be done to determine exactly how the caterpillar was causing the abortions. With this, there was also a very important question needing to be addressed. What is the best way to control the caterpillars? Killing off the caterpillars and their natal black cherry trees, could prove to be disastrous in the future.

What is Happening?

Since the success in lowering the losses due to MRLS in 2003, the search has been on to determine just how the caterpillars are causing MRLS. The winter months after the 2003 foaling season have proven to be no vacation, advancements in the search for the cause of MRLS have been made. There are now two main approaches to solving the MRLS puzzle. One approach is that there is a biological agent, possibly a bacterium or virus, associated with the caterpillar that is causing the fetal losses. The other approach is that there is a toxin or chemical agent that is
in the eastern tent caterpillar and causing MRLS. Both approaches are being taken very seriously and studies to try to eliminate one of them are being done.

In June of 2003, a study was completed that suggested the exoskeleton of the eastern tent caterpillar was associated with the abortions. Eastern tent caterpillars were collected in central Kentucky in the spring of 2003 and stored for the experiment. Thirty-five pregnant mares were divided into seven groups, with five mares in each group. Each group was given an individual treatment for ten days. Mares in group one were fed the caterpillars, and were the positive controls. Group two was the negative control, these mares were given saline. One group was fed just the exoskeleton, another was fed just the gut, and the last two groups were fed the caterpillars that had been homogenized in saline and then separated by size. The results of the experiment were quite conclusive. All five mares in the positive control group aborted. Three of the five mares fed the exoskeleton aborted, and none of the other mares experienced fetal loss. However, the same question still remains to be answered; what is it that is causing the abortions?

Also in June of 2003, there were studies that suggested MRLS was being caused by a non-infectious agent in the caterpillars. The study showed irradiated eastern tent caterpillars inducing late-term fetal loss in pregnant mares. Irradiation has been capable of killing infectious agents such as viruses and bacteria. However, the most recent path that investigations have taken includes the possible involvement of the eastern tent caterpillar’s setae in causing MRLS.

There are studies in progress that suggest that the barbed setae could penetrate the submucosal lining of the digestive tract and release a sort of septic material. The most recent pathological findings show that the caterpillar setae have been found in the lining of the alimentary tract in pigs. The setae have caused microgranulomas and severe inflammatory responses. This could provide a means for bacteria to enter the bloodstream. Following this experiment, a pregnant mare was fed eastern tent caterpillars and fragments resembling the caterpillar’s setae were found in the submucoas of the digestive tract. This is an important finding and is the beginning of controlled experiments done on the mares themselves in determining the role of the caterpillar’s exoskeleton in causing MRLS.

The Future

The future looks bright for understanding the cause of MRLS, however it may remain out of reach for some time. So much remains to be understood, and money for funding of experimental studies is in constant demand. The search for the actual cause of MRLS can be broken down into a few key objectives. The first objective is to identify potential causative agents, and slowly eliminate them until one is left. This can be done by using laboratory models (such as rats and pigs) in laboratory experiments. Another key objective is to determine effective measures to be taken in preventing MRLS from striking again. The last objective is of great debate. Some believe that the complete eradication of the eastern tent caterpillars would prove to be effective. This could end the occurrence of MRLS, but at the same time, could be causing other problems. An effective control of the caterpillars needs to be devised. Suggestions include using a pyrethroid insecticide as a barrier on fence lines to keep the caterpillars from wandering through pastures. Another possible way to control the caterpillars may be to control them by using pheromones. The caterpillars use pheromones to communicate with each other. These pheromones could possibly be used to safely lead the caterpillars away from the pastures and mares.

The equine community must not forget the importance of finding the actual cause of MRLS and determining the most effective measures to prevent it. Some people are concerned that a premature conclusion on the cause of MRLS could be drawn. This could lead to false beliefs and the use of measures deemed preventative, that would not be successful in preventing MRLS.
Mare reproductive loss syndrome hit central Kentucky in 2001 and proved to be absolutely devastating. Since the epidemic in 2001, researchers, veterinarians, and investigators have been scrambling to understand the syndrome and figure out how to prevent it. Many advances have been made, the culprit may be known, but the final chapter in the MRLS mystery remains to be written. More funding is of utmost importance in determining the cause of MRLS and furthermore, how to prevent it from happening again. The financial as well as biological losses from this syndrome are overwhelming and the equine industry collectively needs to finish the job of completely understanding it. The effort to solve the MRLS mystery could arguably be the best example of a cooperative effort of all involved in the equine industry to solve an equine problem of such significance.

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