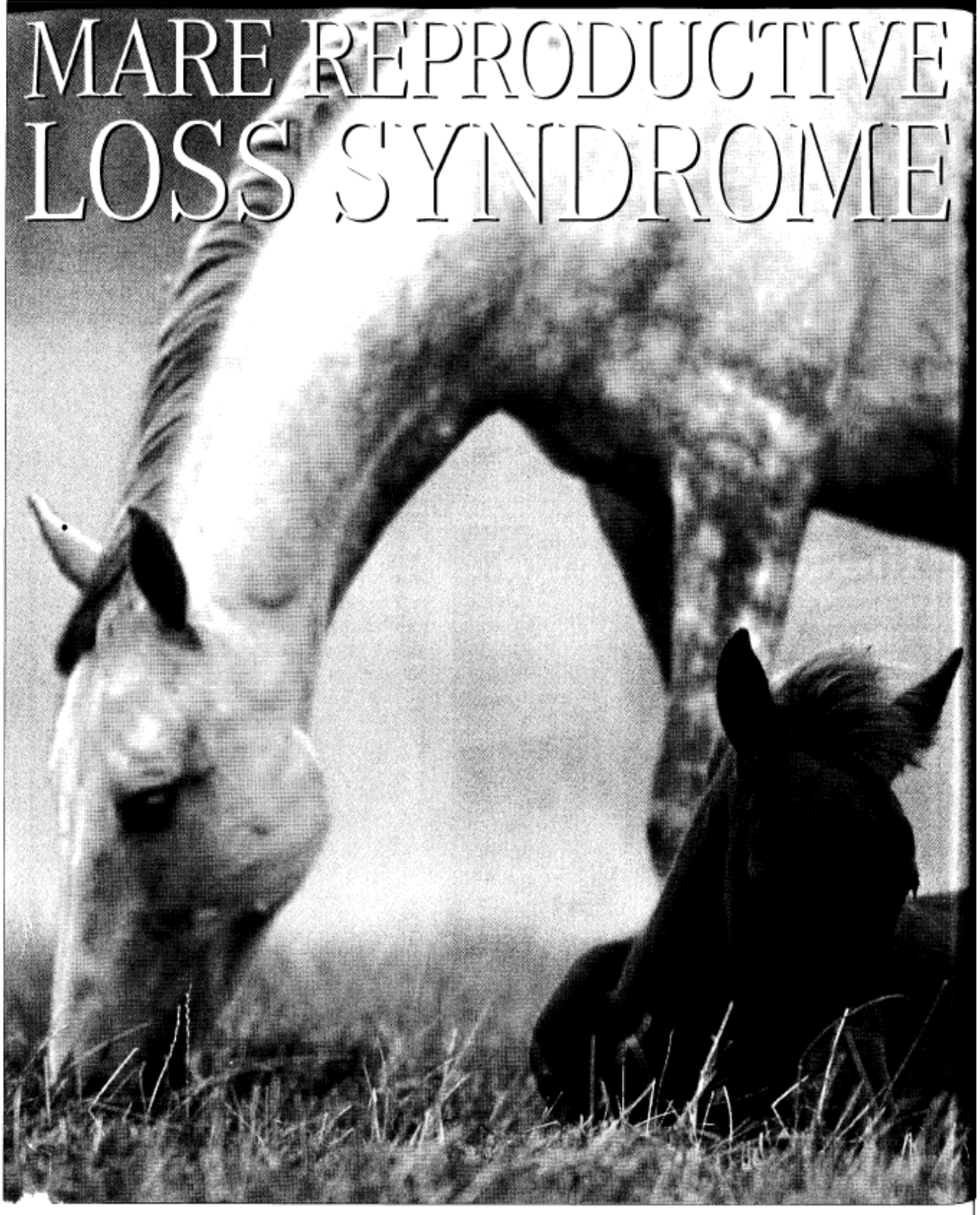
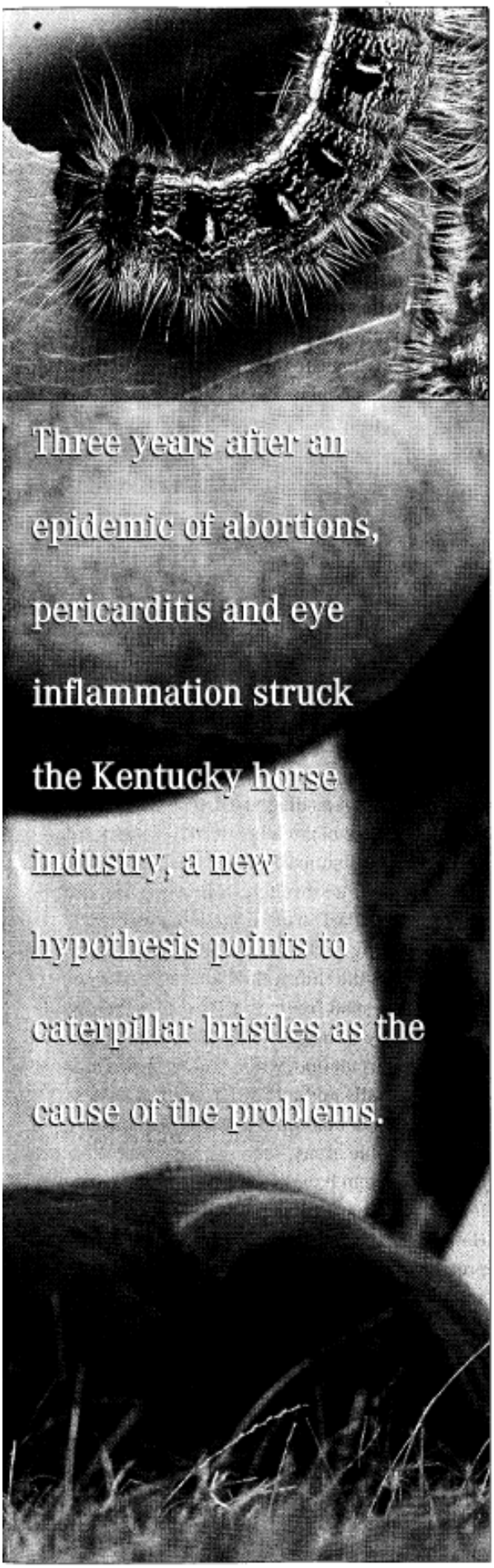
TRACKING THE SOURCE OF MARE REPRODUCTIVE LOSS SYNDROME



ANDI BRONKHORST, T.O. FITZGERALD, PHD (CATERPILLAR)



EQUUS

Three years after an epidemic of abortions, pericarditis and eye inflammation struck the Kentucky horse industry, a new hypothesis points to caterpillar bristles as the cause of the problems.

By Pat Adkins

Death visited the Bluegrass in 2001 during what should have been the season of life. On central Kentucky horse farms an epidemic of spontaneously aborted fetuses and stillborn foals cast a pall over the spring. Some foals born alive were weak and needed intensive nursing care. Breeders counted their losses and feared each day might bring more devastation. By season's end, approximately 17 percent of the pregnant Thoroughbred broodmares in the area had lost foals due in 2001 or 2002. Economic damage to the industry reached an estimated $336 million.

And the disaster wasn't limited to pregnant mares. Adult horses were affected in other ways, though the incidence of problems was considerably lower. Veterinarians found themselves treating an uncommon number of cases of pericarditis (inflammation of the fibrous sac enclosing the heart) and an unusual unilateral uveitis (inflammation of the pigmented structures within the middle portion of the eyeball). Despite intensive treatment, the inflammation invariably progressed to blindness in the single affected eye.

Soon the plague had a name: mare reproductive loss syndrome (MRLS), a designation used for all of its manifestations, including early fetal loss (40 to 120 days gestation), late fetal loss (approximately 220 to 250 days gestation), pericarditis, uveitis and *Actinobacillus* encephalitis, a bacterial infection of the brain.

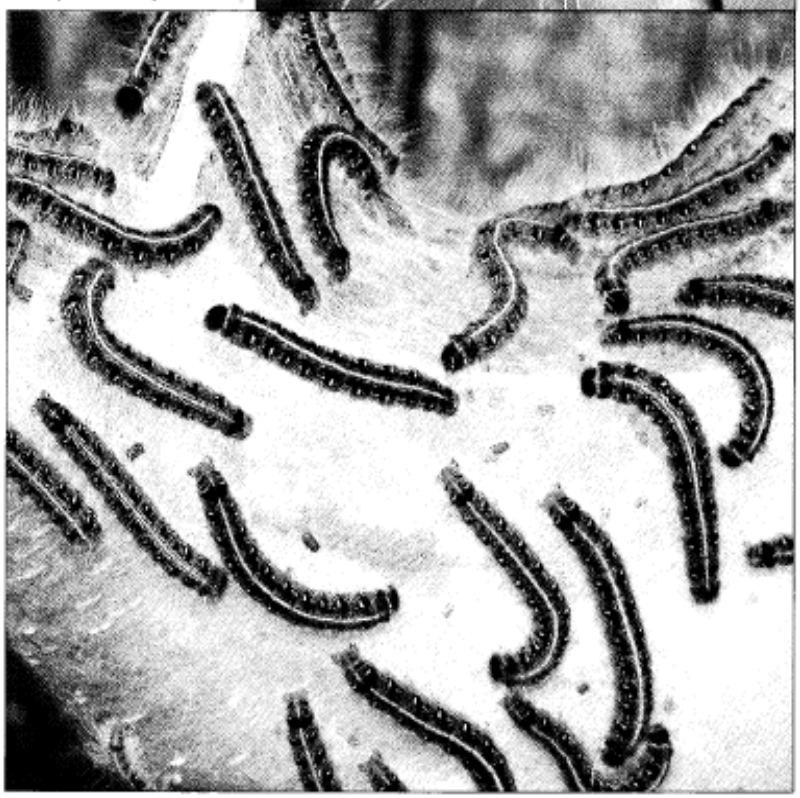
What has remained elusive, however, is the syndrome's precise cause. Initially, a toxin, perhaps in the grass, was suspected. Some looked for bacteria. Others, particularly those who remembered the fetal losses that occurred following a caterpillar outbreak 20 years earlier, suspected a connection to the eastern tent caterpillar, which had appeared in overwhelming numbers across central Kentucky that spring. But even if the caterpillars were to blame, the exact nature of the relationship was the subject of much speculation.

One early toxin theory involving the caterpillars centered on their relationship with the black cherry trees prevalent in the area. The trees' leaves, a favorite food source for the caterpillars, contain cyanide, raising the possibility that the affected horses suffered cyanide poisoning as a result of accidentally ingesting the caterpillars.

Now a group of researchers, led by Thomas Tobin, MVB, PhD, DABT, at the Maxwell H. Gluck

NEW FOCUS: The hairlike setae visible on the eastern tent caterpillar may be responsible for the variety of problems associated with mare reproductive loss syndrome.

POPULATION DENSITY: The eastern tent caterpillars hatched from a single egg mass, which may contain from 150 to 400 eggs, stay together to spin a silken tent amid tree branches and feed upon nearby leaves.



PHOTOS BY T.D. FITZGERALD, PHD

Equine Research Center at the University of Kentucky in Lexington, is proposing a new hypothesis to account for the unusual nature of MRLS. Formally known as the septic penetrating setal hypothesis of MRLS, it suggests that the barbed, hairlike structures known as setae, found on the outside of eastern tent caterpillars, are the source of the problem.

The hypothesis, which has yet to be proven with definitive scientific research, accounts for the possibility that the slender, rigid setae introduce bacterial pathogens into the horse's gut when he inadvertently ingests them. However, Tobin and his colleagues believe the likely explanation is more complex. They think the contaminated setae are capable of migrating from the gut and traveling rapidly via the bloodstream throughout the body to distant sites, including the heart, eye and especially the fetus of a pregnant mare.

Bacterial hitchhikers

According to the researchers' hypothesis, the process begins when a horse ingests the caterpillars. Fragments of the setae pick up bacteria from the horse's oral cavity, typically *Actinobacillus* and nonhemolytic[4] *Streptococcus*. Both are consistently recovered from the umbilical tissue, lungs and placentas of horses diagnosed with MRLS. Then the septic setae begin a potentially devastating journey. Their fishhook-shaped barbs help them to enter and migrate through various moving tissues. This is particularly true in the intestinal tract, where ongoing peristaltic movements propel the setae toward the thin-walled absorptive blood vessels. The setal fragments that enter the vessels travel rapidly through the bloodstream. Some remain in circulation, while others move through the vessel walls and into surrounding tissues.

When small amounts of septic material lodge in less vulnerable areas, such as muscles, the horse's immune system handles the potentially infective intrusion without clinically significant damage. However, some portions of the body are not as well protected by the immune system. Even a small amount of bacterial contamination in amniotic and other extracellular fluid—for instance, the liquid found in the eye or around the heart or brain—quickly leads to serious trouble. Bacteria in the amniotic fluid of mares who have been pregnant for 40 or more days grow rapidly and result in the death and expulsion of the fetus—within as few as 30 hours in experimental MRLS. Pregnancies of fewer than 40 days have not yet established amniotic fluid and, therefore, are less vulnerable.

Using mathematical analysis in the lab

Tobin, whose specialty is toxicology, began working on MRLS by looking for a toxin such as cyanide but concluded that the evidence pointed to something else. He and his group then employed a sophisticated mathematical-analysis technique, known as the accelerated failure time survival model, which is designed to relate events to the time they occur. The technique had not been used previously in equine toxicology.

In the experiments that followed, ground-up eastern tent caterpillars and bacteria were delivered via nasogastric tube directly into the stomachs of pregnant mares, and they aborted without showing other clinical signs. The time that elapsed between the administration of the caterpillars and the abortions was mathematically predictable according to the size of the dose. Abortions occurred in as few as 30 hours if the dose was large enough. As the dose was reduced, the time between administration and abortion increased.

Another element the researchers considered during their trials was the type of bacteria that caused broodmares to abort. The bacteria introduced in the lab were different from the bacteria commonly found in horses who experience MRLS in the field. Yet the infective agents' action was the same: Somehow they traveled from the gut to the amniotic sac, where they caused the mare to abort her fetus. This finding led the researchers to hypothesize that the caterpillar setae are serving as a vehicle for a variety of bacterial hitchhikers.

From skepticism to interest to more research

In the research community, the new hypothesis was met with great skepticism at first, then growing interest. Some questioned whether setal fragments were being ingested until other researchers discovered them in the gut of necropsied horses, pigs and rats used in various studies. Those findings increased the plausibility of the hypothesis proposed by Tobin and his colleagues, although the exact role of the setae continues to be the subject of debate.

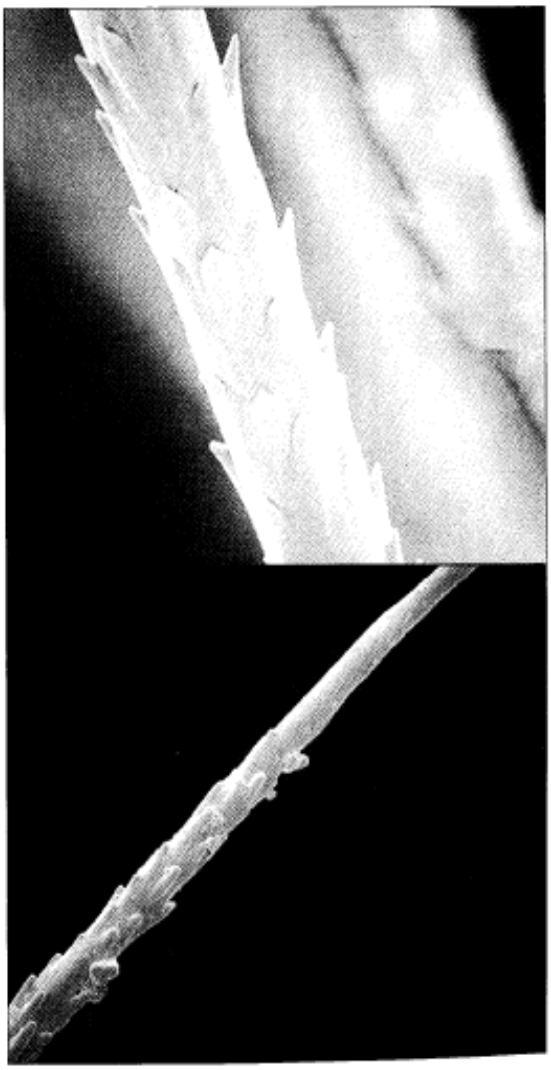
"Tom is insightful in coming up with this hypothesis and championing the idea of more research on it, but there has yet to be a definitive study published that establishes a cause-and-effect relationship," says Terry Fitzgerald, PhD, the entomologist who wrote the book *The Tent Caterpillars,* published by Cornell University in 1995. It was from Fitzgerald, a Distinguished Professor in the Department of Biological Sciences at the State University of New York–Cortland, that

FUNCTION

What are setae for?

Beyond whatever role they play in mare reproductive loss syndrome, the setae of eastern tent caterpillars serve an important purpose: The tiny, bristlelike hairs sense touch. Attached to nerve cells, they relay information about the environment to the caterpillar's brain.

Eastern tent caterpillars aren't the only creatures endowed with setae. The structures also help spiders to hear and to feel, and they are used to pull sticky silk from the spinneret. Earthworms, leeches and other annelids—invertebrate organisms that have a flat body divided into segments—use setae for locomotion and defense. And millions of setae on the bottom of a gecko's feet function as an adhesive, allowing the lizard to climb vertically and travel even when it is upside down.



TROUBLESOME BRISTLES: A microscopic view of a segment of a seta from an eastern tent caterpillar shows the sharp barbs that may help to propel the rigid structure from a horse's digestive tract to vulnerable sites throughout his body. The diameter of the seta (below) is comparable to that of an equine pulmonary capillary.

COURTESY HENRY H. SOUTHGATE AND RICARDO BESSIN

RESOURCES

Read more about MRLS online

Check out the Proceedings of the First Workshop on Mare Reproductive Loss Syndrome on the Web: www.ca.uky.edu/agc/pubs/sr/sr2003-1/sr2003-1.htm. A one-page summary of the hypothesis proposed by Thomas Tobin, MVB, PhD, DABT, and colleagues is available on page 75.

There also are many more details on how the research was performed, the mathematical model used, discussions of the mechanisms involved in moving setal fragments through the body and ideas for researchers interested in testing the hypothesis.

For more on MRLS—including caterpillar control, disease prevention, additional research and archives—go to www.uky.edu/ag/vetscience/mrls/index.htm.

Tobin learned about the nature of eastern tent caterpillars.

"Other researchers, such as Bill Bernard, DVM, Karen McDowell, PhD, and Bruce Webb, PhD, have performed critical studies that established that only the outside of the caterpillar, where the setae occur, causes problems," says Fitzgerald. "However, there is more on the outside of the caterpillar than setae, and these studies didn't establish that the setae were the components that caused the abortions. There seems to be some consensus forming around the idea that the setae affect the gut by introducing bacteria in some way. We need more scientific research."

Some of that research will be conducted at the Gluck Center. With funding from the Kentucky Thoroughbred Owners and Breeders Association, McDowell will study several aspects of the caterpillars' effects on pregnant mares. She plans to use four groups of pregnant mares in her experiments. One group will be given only caterpillar hairs; a second group will receive "polished" caterpillars, from which the hairs have been removed; a third group will receive the entire caterpillar; and a fourth group will be given nothing.

McDowell, whose early research found caterpillar setae in the intestines of pigs, also plans to study whether the mares' immune systems mount a response to the setae. The experiments are expected to yield new insights into the caterpillars and their role in transporting bacteria normally found in horses—but not in the caterpillars themselves—into vulnerable areas within the equine body.

Prevention and treatment

So what does the septic penetrating setal hypothesis mean to horse owners, particularly those involved in breeding? Tobin, Fitzgerald, McDowell and others believe that it helps to reinforce the notion, "no caterpillars, no problem," which was proposed to Tobin early on by Jimmy Henning, assistant extension director for agriculture and natural resources at the University of Kentucky. The hypothesis also indicates the advisability of taking certain precautions, especially during a caterpillar outbreak, when the creatures seem to be everywhere.

Caterpillar infestation can be prevented by removing and destroying the egg masses that encircle tree branches in winter and the tents that the insects spin in spring, and by applying insecticide. Keeping horses off the fields or muzzling them when the caterpillars become apparent can reduce the risk that they'll accidentally ingest any of the creatures' parts. And it's wise, as Fitzgerald notes, to pay attention to the horses' water source, too, because large numbers of caterpillars often collect in troughs.

If a horse, especially a pregnant mare, does come in contact with eastern tent caterpillars, rapid administration of antibiotics may be beneficial. "Our hypothesis offers a justification for the use of antibiotics," Tobin says. "But keeping horses away from the caterpillars is really the key."

Tobin points out that eastern tent caterpillars and similar species occur outside of Kentucky and may well be a little-recognized problem for horses in other areas of the country. He adds that it was the concentration of both very closely monitored pregnant mares and caterpillars in central Kentucky in 2001 that created a medical mystery and set the wheels of research turning.

"I am proud to say that it was all figured out very fast indeed," Tobin says. "The caterpillars were correctly pinpointed within three weeks and appropriate preventive measures put in place. Then, in 2002, within weeks of getting our hands on the next eastern tent caterpillar crop, we may well have pinpointed a unique pathogenic mechanism previously not described in biology or medicine. It was a major team effort, led by deans Scott Smith and Nancy Cox of the College of Agriculture, Dr. Peter Timoney, director of the Gluck Equine Research Center, and Dr. Lenn Harrison, director of the Livestock Diseases Diagnostic Center, with major and unstinting contributions from many equine practitioners and academic researchers. Significant financial support for MRLS research came from the U.S. Department of Agriculture Ag Research Service and Thoroughbred Charities of America."

The caterpillars are sure to return in high numbers some day, which is why all involved emphasize the need for more research. Armed with a newfound and hard-won understanding of the dangers, horse owners can be better prepared to prevent the kind of losses suffered in the spring of 2001.