New Way to Treat SEPTIC JOINT INFECTION IN FOALS

Courtesy Heather Smith Thomas

by Heather Smith Thomas

Research trials are beginning to assess a new process for treating multidrug-resistant bacterial joint infections (septic arthritis) in foals. This research is a partnership between Colorado State University, Ontario Veterinary College in Guelph, and Rood and Riddle Equine Hospital in Kentucky.

Dr. Thomas Koch (Department of Biomedical Sciences, Ontario Veterinary College), founder and CEO of eQcell—a company that is also partnering in this research - has co-authored several papers discussing early research looking at combining antibiotics and stem cells in treating these resistant infections. Mesenchymal stromal cells (MSCs) were used with antibiotics with good results.

Antimicrobial resistance (AMR) has been a growing concern in treating humans as well as animals. Antimicrobial-resistant bacteria are emerging because of excessive antibiotic use—unnecessary treatments, or misuse such as suboptimal dosage or

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At far left, Thomas Koch is the founder and CEO of eQcell, a company that is partnering in this research looking at combining antibiotics and stem cells to treat resistant infections.

At left, Research trials are taking place to assess a new process for treating multidrug-resistant bacterial joint infections (septic arthritis) in foals.

Antimicrobial resistance (AMR) has been a growing concern in treating humans as well as animals. Antimicrobial-resistant bacteria are emerging because of excessive antibiotic use—unnecessary treatments, or misuse such as suboptimal dosage or duration.

duration. People are worried that misuse of antibiotics in animals will hasten development of resistant pathogens and leave fewer options for treatment in humans and animals.

The emergence of "superbugs" resistant to antimicrobial medications threatens animal and human populations due to widespread use of a limited number of antibiotics available for treatments. In recent years, the American Veterinary Medical Association (AVMA), the Federation of Veterinarians of Europe (FVE), and the Canadian Veterinary Medical Association (CVMA) released a joint statement on responsible and judicious use of antimicrobials and published guidelines for appropriate veterinary antimicrobial use. Many federal agencies, in various countries, are moving to reduce the use of antimicrobials in animals.

A different approach must be sought to find alternatives to or enhancements of conventional antimicrobials. Clinicians today may want to consider other strategies for treating microbial infections, and one strategy may include mesenchymal stromal cells (MSCs). These unique cells have been explored in regenerative medicine, for engineering tissues or as immune-stimulating agents for treatment of inflammatory diseases. More recently, MSCs have shown promise as a potential treatment to address infections that are resistant to antimicrobial treatments, Koch points out.

"MSCs have antimicrobial properties; they secrete antimicrobial molecules (peptides) that directly interact with pathogens, as well as other factors (cytokines) that boost antimicrobial activity of the host's own immune cells. In some studies, MSCs have shown strong synergy with standard antibiotic treatments to penetrate biofilm infections, as well as being able to serve as antifungal, antiviral, and anti-parasitic agents," he says.

Mesenchymal stromal cells (MSC) have antimicrobial properties that may help solve the problem of antibiotic-resistant bacteria. "Recent findings in equine, canine, and bovine studies have shown that MSCs have significant effects on a variety of bacterial species either alone or in combination with antibiotics," says Koch.

MSCs exert their effect directly through secretion of various bioactive factors or indirectly through recruitment and activation of host immune cells. MSCs may soon become a valuable tool for veterinarians treating antimicrobial resistant infections, but a great deal of work remains for the development of optimal MSC production conditions, and testing for efficacy on different indications and species.

"The studies at Colorado State University have been going on for a while; I came into this project a bit later, to collaborate with them—but the initial groundwork was done by Drs. Steven Dow and Lynn Pezzanite at CSU," Koch says. Now his company, eQcell, has joined with them to try and push their discovery toward commercialization.

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Dr. Steven Dow, DVM, PhD, DACVIM, Professor at Colorado State University and Director of the Center for Immune and Regenerative Therapies.

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Lynn Pezzanite, DVM, MS, PhD, DACVS-LA, Assistant Professor, Colorado State University College of Veterinary Medicine.

EARLY STUDIES WITH MICE

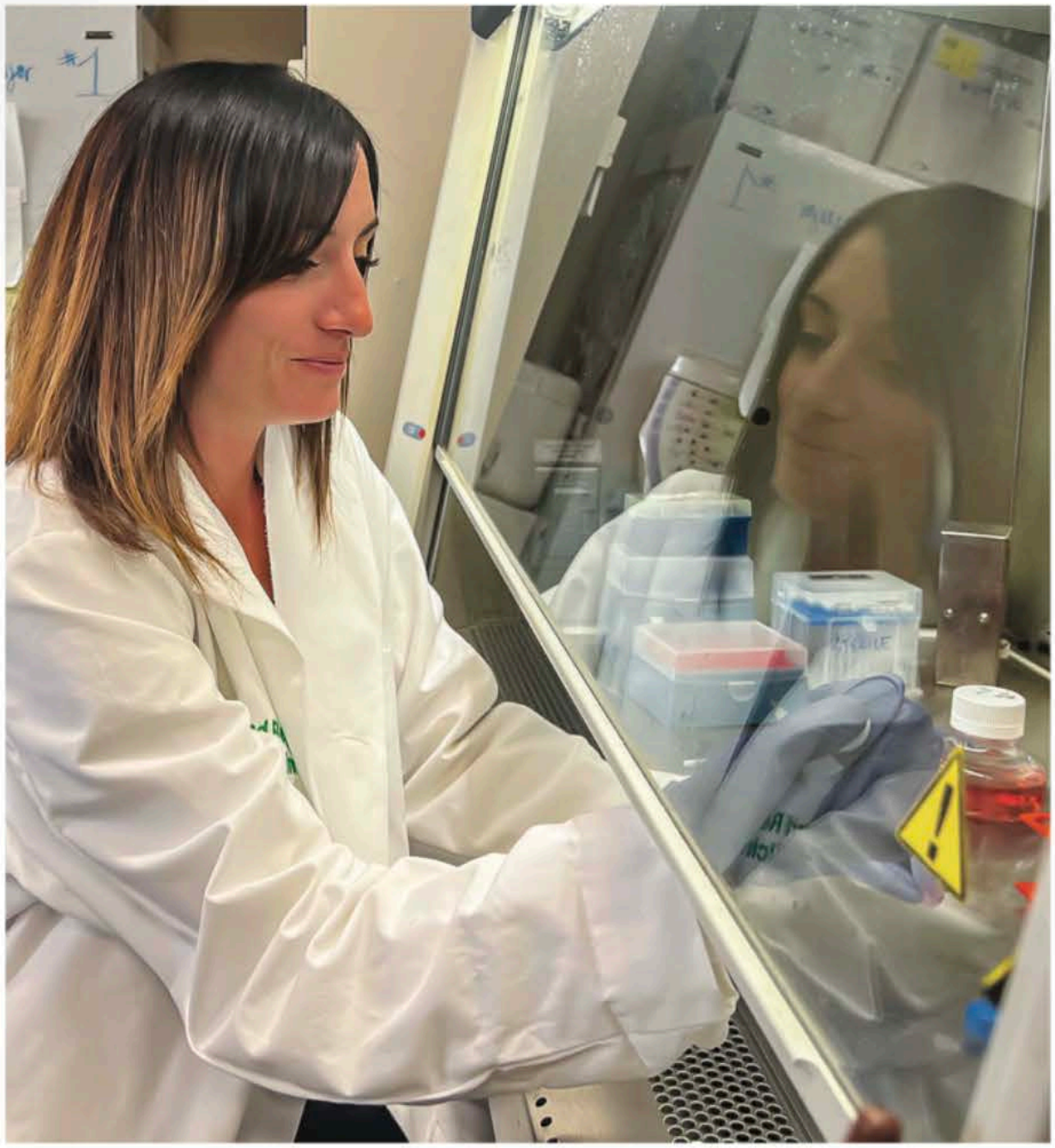
"They have done some very interesting work, both in dogs and in horses. They started out, however, with mice. They made skin wounds in mice and then infected those wounds with multi-drug resistant bacteria. Then they treated the mice in different ways," he says.

Some mice were left untreated, and some were treated with either antibiotics alone or stem cells alone. Another group was treated with manipulated stem cell, activated in culture to be more antibacterial. "Then they treated some of the mice with combinations of either stem cells and antibiotics or activated stem cells and antibiotics," Koch says.

"Among all the mice, they saw the best response (clearing up infections) in the mice that received the antibiotic plus the manipulated stem cells. With this combination there was a synergistic effect with the MSCs, compared with antibiotic alone," he says.

"The stem cells alone, without the antibiotic, were not good enough, and the antibiotic alone was not as effective." So, they determined that the manipulated MSCs could be utilized as an add-on treatment with antibiotics to improve infection control.

"You still need to treat the infection with antibiotics, but if you add the stem cells there is a better response than with the antibiotics alone. It is not completely clear what the underlying mechanism is, but there is a synergistic effect with the combination."



Courtesy Heather Smith Th

In early studies with mice, some were untreated, others were treated with either antibiotics alone or stem cells alone, while another group was treated with manipulated stem cells activated in culture to be more antibacterial, and these mice saw the best response of clearing up infections.

RESEARCH IN DOGS

Then the researchers treated seven dogs at the CSU teaching hospital. "These were client-owned dogs that had documented bacterial infections with multi-drug resistance. These were non-responders; they were not responding to the prescribed antibiotic treatments even though the veterinarians were using very potent antibiotics," says Koch.

Some of these dogs had infections varying in duration from several months to almost two years. "They had different types of conditions. Some of them had deep skin infections in their paws. Some infections were the result of surgical implants and other situations. The infections were quite varied, but they were all deep-seated and long-lasting despite treatment," he explains.

"They treated these dogs three times, two weeks apart, with intravenous infusion of manipulated stem cells. In five of the seven dogs they saw a complete cure; the infection cleared up and the dogs became healthy, without any signs. Bacterial testing and culture sensitivity showed that the infection was no longer there."

The other two dogs were partial responders. There was some improvement, but not a complete cure. "The results were exciting from a veterinary perspective, but also the fact that this new strategy holds a lot of promise for human medicine," he says.

Dr. Steven Dow, DVM, PhD, DACVIM, Professor at Colorado State University and Director of the Center for Immune and Regenerative Therapies, has now treated many dogs successfully at the vet school using this combination therapy. "These were dogs with chronic drug-resistant infections. We know that it works in larger animals, and not just mice. We've treated more than 20 dogs and have a good level of understanding about this therapy and a good level of comfort using this to treat dogs," Dow says.

"They were people's pets with a variety of infections that had not responded to treatment for at least a month. Some were joint infections, some were soft tissue, and some were bone infections. They had all been treated with the various drugs used here in the clinic," he explains.

The results were exciting. "This is why we are enthusiastic about this new strategy for treatment, and it is also very relevant to treating humans. The types of infections in the dogs, and the body sizes, are not that different."

RESEARCH WITH HORSES

"A study to look into this with horses was funded by the Grayson Jockey Club using research horses," says Koch. Normal horse joints were infected with a USA300 MRSA superbug bacterium that had been isolated from a human patient and donated to the research group. Those joints became infected, and the horses became lame.

"Then they treated some of these infected joints with antibiotic alone—one that they would normally choose, clinically, and expected to work against those bacteria based on culture and sensitivity. A second group of

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horses were treated with the same antibiotic plus manipulated stem cells. They have seen a significant improvement in joints treated with manipulated MSC and antibiotics in degree of lameness and other outcome parameters they measured including reduced bacterial burden and inflammatory cytokine levels. Overall, there was a significant effect when adding the manipulated stem cells to the treatment protocol," Koch says.

"The horses that got the combination cleared the infection, were less lame and had a better treatment response." This preliminary effort paved the way to move into treating client-owned horses in order to assess whether adding stem cells to antibiotics will have an effect in those horses with naturally occurring, rather than induced, bacterial infections in joints.

Lynn Pezzanite, DVM, MS, PhD, DACVS-LA (Assistant Professor, Colorado State University College of Veterinary Medicine), had the opportunity to start her PhD project in Dr. Dow's lab. "Some of the initial groundwork with the in vitro human MSCs and initial mouse studies were already ongoing in Dow lab when I began my graduate program," Pezzanite says. The mouse studies were comparing the different MSC and antibiotic combinations regarding effectiveness in treatment.

"Part of my thesis program was funded by an NIH (National Institutes of Health) program in partnership with the UC Denver medical school, with the goal of encouraging communication between the veterinary and medical school programs. One of Dr. Dow's collaborators, Jason Stoneback, Chief of Orthopedic Surgery and Director of the Limb Restoration Program at the medical school, was my mentor through the program. He works with challenging human orthopedic cases, which present many of the same difficulties in treatment as they are often complicated by infection with multidrug-resistant bacteria. He was interested in looking at this kind of treatment for humans as well. The use of our anti-infective cellular therapies in larger animal models, dogs and horses, provides further support for integration of these therapies in human clinical trials, moving from mouse models and in vitro work," she says.

"Treating horses with infection is my main focus here at the veterinary school, but our findings also represent a translational model for humans suffering from the same processes." She has submitted a paper for publication describing the work she's done using an equine model, looking at septic arthritis in adult horses. Her work has shown that the treatment can work very effectively.

"In adult horses, we've seen more rapid improvement, both in clinical parameters and lameness and in their overall pain/inflammation scores—in the horses treated with stem cells and antibiotics versus just antibiotics alone," she says. "Some of our in vitro outcomes were also exciting in terms of how these cells work. While there is certainly more to learn in terms of how these cells are exerting their effects, our study findings in adult horses with joint infections demonstrated that those treated with stem cells had lower levels of inflammatory cytokines and more rapid healing/normalization of joint fluid parameters, including white cell count and inflammatory mediator serum amyloid A. They also had lower bacterial bioburden in their joints based on quantitative cultures compared to horses that received antibiotics alone," she says.

NEW STUDY WITH FOALS

Dr. Scott Hopper at Rood and Riddle Equine Hospital in Kentucky is involved in the study with foals. "Their practice frequently treats Thoroughbred foals that are intended to be racehorses. Foals often encounter a number of systemic diseases in their early months of life. These include colitis, umbilical and respiratory issues, and all of these can get into the bloodstream and seed infections into the joints. Thus, they are very susceptible to joint infections," Pezzanite says.

"This study will employ the same techniques that Dr. Dow pioneered in the lab and what we did in adult horses, using activated stem cells in combination with antibiotics in the joint itself." Foals in the study will be randomized into groups. Some will receive the standard treatment of lavaging and debriding the joint, and others will receive all standard treatments and this new treatment of stem cells in addition. "This will give a comparison to see if this combination is more effective than standard treatments."

Joint infections, especially those that don't respond to standard treatments, can leave the joint permanently damaged, which is detrimental to a young horse intended to be an athlete. If they develop arthritis secondary to infection and are lame, they won't be able to have an athletic career. This new treatment will be an exciting improvement if it works for these foals, not only hopefully to improve treatment of the infection initially but also potentially to improve long-term athletic outcomes in these young athletes.

"A large part of our interest and enthusiasm for using this in foals is knowing that it works in dogs—which are more the size of a foal than the earlier mouse models," says Dow. "We can probably use doses equivalent to what we've been using in dogs. We also know that infusions of these activated stem cells are very safe."

Some adult horses with puncture wounds into a joint or some other injury get non-responsive long-standing infections, but the researchers have chosen to start their new study with foals less than six months of age. "These are foals that have been diagnosed with joint infections which may be due to infections in other areas of the body, such as the intestine or umbilicus," Koch explains.

Sometimes foals have more than one joint infected if the bacteria have spread through the bloodstream. A foal may have bacterial invasion that enters via the navel stump, the lungs, or digestive tract, getting into the bloodstream to cause a bacteremia or even a septicemia. That's how it gets seeded into the joints—affecting more than one joint. "If it's a foal with multiple joints affected, all of those joints will get the stem cells if that foal is allocated to the stem cell treatment group," he explains.

"We have selected four different antibiotics that can be used. They were chosen by the researchers in Colorado after evaluating a number of antibiotics to find out which ones the stem cells can tolerate. Some antibiotics are toxic to them and will actually kill stem cells. They have screened a number of antibiotics to determine their degree of cell toxicity. Based on that, we've reduced it to a short list of four antibiotics that should not affect the cells too badly; the cells should not be killed by these antibiotics," he says.

"We have a hierarchy of treatment for this study. First, we will use a certain antibiotic, and if that fails to make a difference they can go on to another antibiotic, and so on. There will be foals that only receive antibiotics and they will

In a study to accelerate the healing of joint infections in foals, the control group only receives antibiotic - then there will be foals that receive that same antibiotic plus stem cells (shown).

be our control group. Then there will be foals that receive that same antibiotic plus stem cells.

This study is being conducted at three different locations. "Foals that come to Colorado State University with joint infections will be treated at their teaching hospital. Foals will also be treated at the Ontario Veterinary College Teaching Hospital and also at Rood and Riddle Equine Hospital in Kentucky. We expect that the majority of foals will be enrolled at Rood and Riddle because the teaching hospitals at universities don't see as many foals," says Koch.

This study is just getting started. "We are growing cells in the lab now and will ship them to Rood and Riddle, and they will start to enroll foals sometime in May. Dr. Scott Hopper at Rood and Riddle is the equine surgeon there who is involved in the study and doing the planning for it, putting together the protocol for it; some of his colleagues at that hospital will also be treating foals."

This new strategy for treating foals with septic arthritis is exciting since these types of infections can be very detrimental to a foal's potential athletic career. "It is an exciting project, not only because of the need for better treatment, but also because this project has some data—both in dogs, mice, and horses—to support this approach prior to starting. We are eager to get it underway," Koch says.

"As a company involved with stem cells, we would like to establish this type of partnership with people in academia who discover methods that lend themselves to commercialization to help more patients. eQcell wants to be that partner company to further develop a product and get it to market. We have a very good relationship with the researchers in Colorado, and they chose us to push their discovery forward toward market approval, and they are very excited about that," he says. This is a unique treatment strategy that has a lot of potential.

It's hoped that this new treatment will accelerate the healing of joint infections in foals. "The cell treatment that's given in conjunction with antibiotics won't replace the antibiotic; they work together," says Dow. "If the foal study gives positive results, we will be looking to also deliver the cells intravenously, rather than just injected into the joint. The studies with dogs were all done with the cells administered intravenously. We showed in the mouse studies that the cells given intravenously can find their way through the body to sites of inflammation or infections. Giving the cells intravenously is a lot easier to do than to inject them into the joint," Dow says.

The joint injections work very well, however, in the adult horses that have been treated. "It's still a very good option, but could be combined with IV delivery, and in other types of infections we would just start with IV delivery."

This new treatment will be a way to deal with drug-resistant infections and will also help clear the joint infection before the joint tissue—especially cartilage—is injured. Then the foals have a better chance to return to full function, and a chance to grow up to be athletes. (S)

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