Zweig Memorial Fund News Capsule

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Learning More About Potomac Horse Fever

Dr. Atwill and Dr. Mohammed

For the past eleven years veterinarians have been baffled by a disease first discovered on horse farms in Maryland, near the Potomac River. This acute infection, which came to be called Potomac Horse Fever (PHF),can infect up to 20 to 30 percent of horses in areas where it is most highly endemic, and has been diagnosed in 32 states as far north as Alaska and right here in New York.

The initial symptoms of PHF are rapid and severe: and may include loss of appetite, high fever, profuse diarrhea, and, sometimes, abortion. In up to 30 percent of unvaccinated horses, death may occur within 24 hours after the onset of the disease . Those that recover can be rendered useless through the subsequent development of laminitis.

All that is known, for sure, about PHF is the organism that causes it—*Ehrlichia risticii.* How horses become infected, the key to controlling and eventually eradicating PHF, remains a mystery. To bring this widespread and costly disease under control researchers at Cornell's College of Veterinary Medicine are turning to New York State's horse owners and trainers for help.

With a grant from the Zweig Memorial Fund, Rob Atwill DVM, MPVM, a graduate student in the Department of Clinical Sciences and Hussni 0. Mohammed, MPVM, Ph.D., an assistant professor of epidemiology, are undertaking a statewide study of the distribution and risk factors associated with PHF. The success of the study depends on how the managers of just 3,000 out of the approximately 40,000 New York horse operations respond to letters they received late this spring.

In the letters, sent with the assistance of the New York Agricultural Statistics Service, Atwill makes a simple request: that owners complete a checklist style questionnaire and that they provide a blood sample from their horses. The sample can be drawn by their own veterinarian or, free of charge, by a veterinarian sent from the university. No other commitment of time or resources is required. And, of course, all of the information provided will be kept strictly confidential.

In return for their help, the results of the laboratory analysis of the blood samples (tested only for the presence of Ehrlichia risticii antibodies) will be provided to participants as quickly as possible. In addition, a summary of the study's findings will be sent early next winter.

Drs. Atwill and Mohammed emphasize that the answers to the checklist provide invaluable information—information obtainable no other way. "When PHF was first discovered researchers had hoped to figure out how horses became infected by studying a few individual farms where outbreaks of the disease occurred," Dr. Atwill explains. "This approach has often worked in the past but, unfortunately, it did not work with PHF. New clues may be provided on how horses are becoming infected with PHF by attaining a comprehensive, state-wide picture of the disease. We can't do this alone."

The blood samples are needed to determine those areas where PHF is present. While some horses who contract it exhibit blatant symptoms, in others the symptoms are very mild. "It's possible that an owner had several infected horses

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and didn't know it," Atwill points out. Also the presence of infection could explain the cause of abortion, whereas typically the cause is never determined.

What Drs. Atwill and Mohammed are looking for is common denominators—that is facts that keep turning up again *and again* when the disease is present. The answers to simple questions such as whether horses are stalled or pastured, and the location of an operation (near a river or up in the mountains, for example) could provide the critical clues needed to point the researchers in the right direction. Right now, it seems as if river valleys are a "hot spot" for this disease.

One of the most puzzling aspects of PHF is that there are some valleys in New York State where horses are plagued by it year after year, while in other valleys it rarely occurs. By surveying horse operations in every county Atwill and Mohammed will get a picture of where the "hot spots" are, that is, where repeated or substantial outbreaks occur. Then they will use this information to follow-up the strongest lead they have on how the disease is transmitted.

"Researchers early on noticed that PHF usually occurs from late spring to late fall, the time of year when insects are prevalent," explains Atwill. "Also there is a similar disease in dogs, *Ehrlichia caninus*, that is known to be passed by ticks." So one of the first things Atwill and Mohammed will do when all the questionnaires have been returned is make a map of "hot spots" throughout the state.

If certain insect populations exist where PHF is common Atwill and Mohammed will then examine these insects for the presence of *Ehrlichia risticii*.

At present, horses in New York State are often vaccinated twice a year against a possible infection with PHF. Antibiotic treatment can be expensive. "This study gives us the chance to learn more about PHF," says Mohammed. "In the short run it will help us to come up with better ways to prevent the disease."

What's more, Atwill adds, it's an opportunity to establish a spirit of trust and cooperation between the College and horse managers in the state. "We want people to know we are interested in practical problems, that we want to solve them by working together."

The Prevalence of Potomac Horse Fever in New York State, 1985-1986

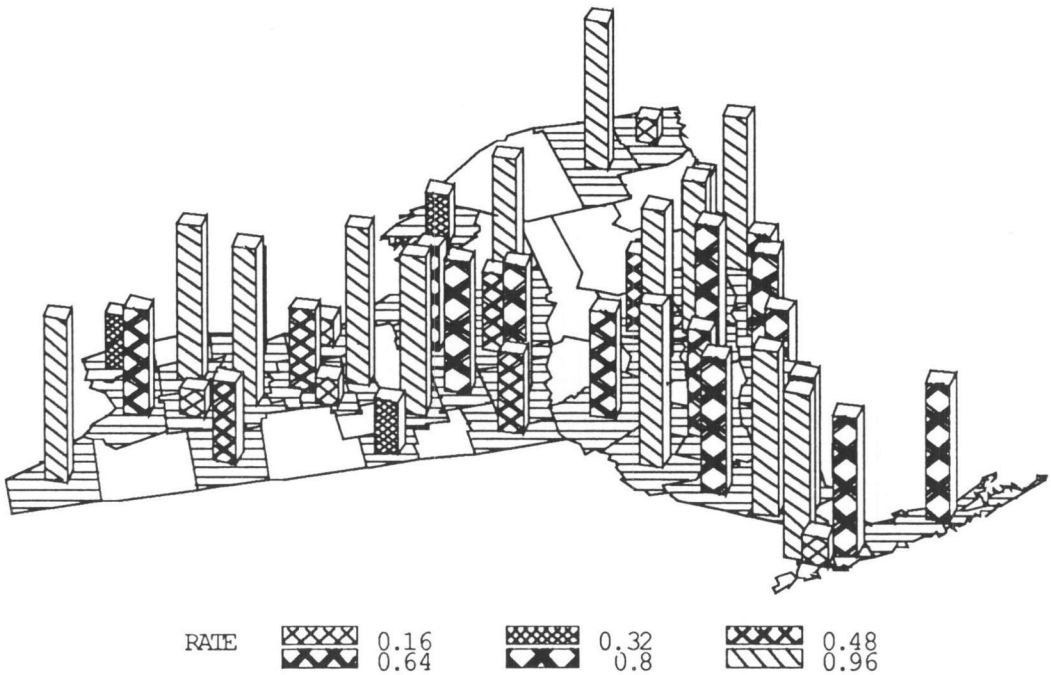

Figure shows the distribution of Potomac Horse Fever in New York state by county. This disease distribution is generated from a retrospective study.

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DEVELOPING A RELIABLE VACCINE AGAINST STRANGLES

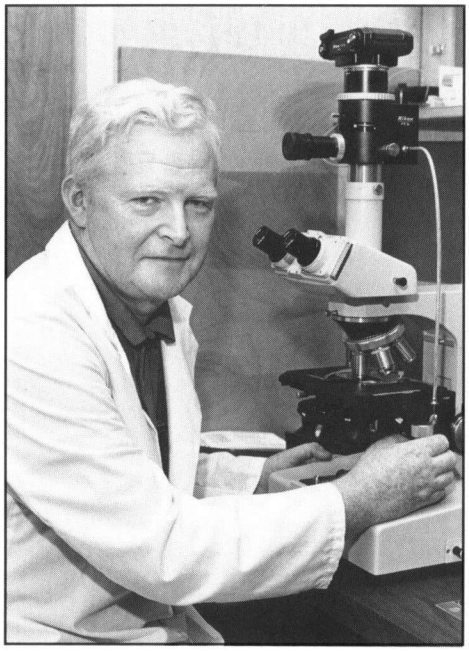
John F. Timoney is in hot pursuit of just the right amino acid sequence. Once he's found it, and has figured out what it looks like, a scourge of the racing industry can be eradicated. Horses will no longer be defenseless against strangles, and the other diseases caused by *Streptococcus equi.*

At present strangles is the most common bacterial disease in the horse. This highly contagious upper respiratory tract infection causes abscesses in the lymph nodes to obstruct the upper airway, resulting in difficult breathing and swallowing. Because of the debilitating nature of strangles a horse can lose a season of racing or the opportunity to be sold. One to two percent of the horses who contract it die.

Although many horses who recover from strangles are resistant to another episode, 30 percent do not develop their own protective immune response. And the commercial vaccines against *S. equi* have proven unreliable. Along with a team of researchers from Cornell's College of Veterinary Medicine, Timoney, a professor of veterinary bacteriology, is investigating how to protect horses from the highly virulent *S. equi* bacteria. Doing so will pave the way for developing dependable vaccines.

Timoney began by pursuing the answers to three basic questions: how the bacteria enters the horse, how it causes disease, and how the horse makes a protective immune response. One of the team's early discoveries is that the entry point of *S. equi* is through the horse's tonsil. This offers an explanation for why commercial vaccines aren't very effective.

"The work in our laboratory has shown that these vaccines stimulate production of serum antibodies (antibodies in the horse's blood) but not local antibodies in the nasopharynx—the posterior nose and throat area of the animal," Timoney explains. "These localized antibodies are crucial to preventing entry of the organism into the deeper tissues and draining lymph nodes."



Dr. John F. Timoney

Once the *S. equi* has invaded the animal's body, Timoney found that its virulence is due to a protein located on its surface, called the M protein. This protein, shaped like two springs coiled around each other hanging out from the edge of the bacteria, has the capacity to circumvent the horse's natural defenses.

These defenses, known as phagocytes, are killer cells which engulf and destroy bacteria in the tissues. The M protein prevents this from happening. "We found that's the strategy the *S. equi* has for allowing itself to survive," says Timoney. When the bacteria survive in sufficient numbers, they multiply in the lymph nodes and abscesses result.

How does the M protein foil the phagocytes? This is where the amino acid sequence comes in. About 500 amino acids of 20 different types are located in different regions along the two strands of the M protein. Timoney believes that a certain sequence of these amino acids (called a peptide structure) located in a particular region interferes with the functioning of an immune system mechanism known as complement.

Complement is a protein in the horse's blood. Ordinarily one component of complement, known as C3b, coats the surface of invading bacteria. This coating is appealing to the phagocytes which ingest it, engulfing the rest of the bacteria. It happens that another component of complement, the H control factor that regulates the production of active C3b, is inclined to stick to a specific peptide structure of the M protein. When it does so C3b is no longer active. Without a C3b coating the phagocyte will no longer ingest the bacteria. This is known as the antiphagocytic effect of the M protein.

But the M protein also has it good points. Timoney found that at a different point on the M protein strand, there is another peptide structure which can stimulate the production of an antibody capable of neutralizing the antiphagocytic effect, thereby allowing the horse's natural defenses to operate effectively. And he found yet a third site where a different peptide structure can stimulate the production of antibodies in the mucous membrane lining the animal's nose and throat.

It became clear, then, that the way to protect the horse from strangles is to harness the antibody stimulating capability of the M protein. To do so requires understanding the protein's structure by separating off fragments, then identifying the sequence of the amino acids found there. Much of Timoney's recent work has been doing just that. It is a laborious and painstaking task.

"No one has ever done this before," Timoney points out, "so much of the time has been spent learning how to do it." One of the biggest problems is that there is very little M protein on the sur-

face of the *S. equi* bacteria—1 to 2 milligrams of it on 5 to 6 grams of bacteria . The researchers have to grow up to 12 liters of *S. equi* to get enough M protein to get the sequence of just one fragment.

"If we're lucky we get 30 of the 500 animo acids in one fragment," says Timoney, "but we usually get only 12 to 15 each time. This process of purifying the protein and getting one fragment for sequencing takes three to four weeks, if all goes well." Many times it doesn't and the researchers must start all over again.

Using computer-assisted technology the team need not characterize all 500 amino acids and their sequence but only those groupings which prove likely to stimulate the right antibody. "Once we know the nature of these amino acid sequences then we can make synthetic replicas of those peptides and possibly have an effective vaccine," says Timoney.

Some of these synthetic peptides have already been made and successfully tested for reactivity with sera of horses who have recovered from strangles. Timoney estimates that it will take another two years before all the needed peptides will be ready.

At that point two different types of vaccine can be developed. One will package the peptide with another sticker molecule in the form of a nasal spray. "When sprayed into the nose of the horse the peptides would stick there, stimulating antibody production," says Timoney. "When *S. equi* come along the antibodies would bind with the M protein thus preventing the bacteria from moving through the tonsil into the interior tissues of the throat."

A second type of vaccine, administered by injection, would protect the horse from *S. equi* already in the tissues, by stimulating the antibody that neutralizes the antiphagocytic effect.

In addition to the vaccines, Timoney's work will enable the development of a test—much like the strep culture done on humans—which will show whether a horse has been vaccinated against *S. equi*, if the vaccination was effective, and whether antibodies are present as a result of previously contracting the disease.

Timoney's work leading to the control of strangles is the only research endeavor of its kind being conducted in the United States. It is supported through a grant from the Zweig Memorial Fund.

The Harry M. Zweig Memorial Fund honors the late Dr. Harry M. Zweig, a distinguished veterinarian, and his numerous contributions to the state's equine industry. In 1979, by an amendment to the pari-mutuel revenue laws, the New York State legislature created the Harry M. Zweig Memorial Fund for the promotion of equine research at the College of Veterinary Medicine, Cornell University. The Harry M. Zweig committee is established for the purpose of administering the funds and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers and veterinarians. Current committee members are Daniel J. Burke, Longford Farm; Donald G. Butcher, former Commissioner of the New York State Department of Agriculture and Markets; Richard Corbisiero, Jr., Chairman, New York State Racing and Wagering Board; John L. Hardy, Tucker and Hardy Associates; Charles Knauss, Jr., Executive Director, Agriculture and New York State Horse Breeding Development Fund; Albert W. Miller, DVM; Everett Schoenborn, Climax, New York; Patricia Wehle, Scottsville, New York; William H. Welch, Executive Administrator, New York State Thoroughbred Breeding and Development Fund; Theodore J. Zornow, Avon Farms; Anna Zweig, widow of Dr. Zweig; and Robert D. Phemister, Dean of the College of Veterinary Medicine, Cornell University, who serves as chairman of the Committee. The Zweig Fund receives two percent of all monies accruing to the Agriculture and New York State Horse Breeding Development Fund and the New York State Thoroughbred Breeding and Development Fund from the state's tracks and off-track betting.

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