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COMMENTARY

IN THIS ISSUE

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LLOYD'S

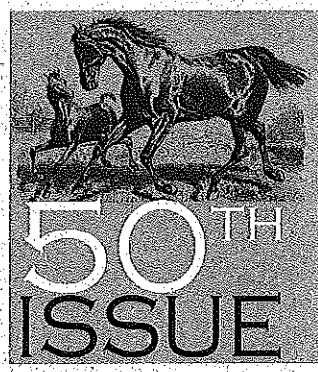
THIS ISSUE MARKS THE 50TH PUBLICATION of the *Equine Disease Quarterly* since its inception in October 1992. An initial circulation of several hundred copies has grown to 17,000, reaching 87 countries around the world. The *Equine Disease Quarterly* is also available on the Internet, and articles are regularly abstracted by a variety of scientific and lay equine publications.

All this would not have been possible without the steadfast financial support of Underwriters at Lloyd's, London, Brokers and their Kentucky agents. Their support of the Department of Veterinary Science at the University of Kentucky began in 1986. It was then, over a three-year period, Lloyd's donated $150,000 to a field study investigating the cause of foal diarrhea, which during the mid-'80s was a major cause of morbidity among the expanding foal population of Central Kentucky. The finding of rotavirus as the primary cause culminated in 1996 with the conditional licensing of an inactivated rotavirus vaccine. The vaccine given to mares during the latter stages of gestation provides passive antibody protection to foals via the colostrum. The vaccine continues to be used on farms in Central Kentucky and abroad.

The funding of this study and the resultant vaccine was followed in 1989 by support amounting to $90,000 over three years for a fellowship in equine pathology at the Livestock Disease Diagnostic Center. Beginning in 1992, Lloyd's initiated funding of the *Equine Disease Quarterly*, currently contributing $40,000 annually to the cost of production.

Since 1986 Lloyd's has donated a total of $640,000 to support various activities undertaken within the Department of Veterinary Science. The outcome of this partnership has provided tangible benefits to the equine industry, not just locally, but at a national and international level. It is earnestly hoped that this support will continue.

To commemorate the 50th issue, the format has been changed to include brief reviews of the research activities undertaken by the five endowed chairs currently occupied at the Maxwell H. Gluck Equine Research Center. Their reports indicate the emphasis on equine infectious disease research, including virological studies of equine infectious anemia and equine viral arteritis, bacteriological studies of strangles and leptospira infections, and studies of the immunology of both young and old animals. One of the most recently occupied chairs has initiated a program investigating genomic aspects of orthopedic problems.



These reports reveal the progress that has been made and the practical implications of the studies for the equine industry, not just at a local level but nationally and internationally. They also reveal the future direction the endowed chairs' investigations are likely to take. Each of the programs encompasses the training of young graduate students from within the United States and abroad in the complex discipline of scientific research and investigation. On graduating with an advanced degree, many of these young highly motivated individuals continue to devote their careers to equine research in institutions around the world. In doing so, they provide the core of scientists essential to undertake the research to improve the health and welfare of horses across breeds, ages, and sexes.

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Equine Immunology: Past, Present, and Future

THE HORSE HAS PLAYED AN IMPORTANT role in the history of immunology, dating back to Emil von Behring's description of curative antibodies in equine serum more than a century ago. Since then, the field of immunology has seen a tremendous increase in information regarding the role antibodies, lymphocytes, and other cells play in a variety of diseases. While the mouse has replaced the horse as the predominant experimental animal in immunology research, efforts are still under way to characterize the immune response of horses. We have a better understanding of the horses' immune system and the role it plays in a variety of infectious and non-infectious diseases, but we trail our colleagues working with mice and other domestic species in terms of both basic and applied information. Current efforts are focused on developing the reagents and techniques necessary for studying the horse's immune system and the characterization of both protective and pathologic responses. As this knowledge base continues to expand, we can anticipate the translation of this basic information into practical application in the clinics, including improved diagnostic and therapeutic applications.

One example of this effort in my laboratory is a project investigating the immune responsiveness of very young foals. The development of immunological responses in newborn animals of all species, including the horse, is poorly understood. Clinical evidence suggests that very young foals are susceptible to a number of bacterial and viral infections despite the presence of maternal immunoglobulins. The unique susceptibility of young foals to infection with *Rhodococcus equi* is a well-recognized example of this age-dependent phenomenon. While resistance to *R. equi* infections likely involved multiple factors, it is known that cell-mediated immunity plays a critical role in immunity to this bacterium. We have recently shown that young foals have deficient cell-mediated immune responses when compared to older foals and adults. This defect is most apparent in foals less than 1 month of age, though foals older than 6 months can still be deficient when compared to adults. The underlying cause of this deficiency is unknown. The process whereby the young foal's immune system matures to the point of being resistant to this bacterium is also unknown. Our efforts are directed towards identifying the nature of this immune defect, understanding the process involved in the attainment of full immunologic function in the foal, and identifying prophylactic or therapeutic strategies that may be employed to enhance the immune function of young foals.

A second project is directed towards characterizing the immune system of older horses. Horses over 20 years of age constitute about 15% of the equine population, and many remain actively involved in equestrian sports and reproductive capacities as stallions and broodmares. Advancing age in horses, as with other species, is eventually associated with a decline in body condition, muscle tone, and immune function. Nevertheless, there are no specific recommendations regarding the vaccination of older horses and ponies, even though the best characterized effect of aging in horses is a reduced response to vaccination. We are in the process of characterizing the mechanism of the age-related decline in immune function in older horses. Once the underlying mechanism is understood, possible approaches for overcoming this effect may then be developed.

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Research into Equine Bacterial Disease

THE DESIRED IMPACTS OF MY RESEARCH program into equine bacterial disease are the development and application of effective and safe vaccines, sensitive and specific diagnostics, and improved understanding of epizootiologic features of value in prevention and management of outbreaks. Success in these efforts requires identification and molecular characterization of bacterial components involved in virulence and protective immune responses, elucidation of mechanisms and modes of induction of protective immunity, and analysis of the host-parasite interaction at the microscopic and ultra structural levels.

STREPTOCOCCAL DISEASES

The need for a more effective and safe vaccine against equine strangles has driven our efforts to identify proteins of *Streptococcus equi* that

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stimulate protective immune responses. The nearly complete genomic sequence of *S. equi* (Sanger, http://www.sanger.ac.uk) has greatly accelerated the pace of this discovery, and we are now evaluating a large number of novel surface-exposed or secreted proteins in immunization/challenge trials and in experimental infections with defined mutants. Comprehensive morphologic and histochemical studies of Dr. Pawan Kumar, visiting Clay Fellow, on the equine tonsillar complex have provided key information on entry of *S. equi* and on the location and stage of infection at which protective immunity is active in resistant horses. Since immunity to strangles is not stimulated by *S. zooepidemicus*, an organism almost identical to the clonal *S. equi*, Sergey Artiushin is sequencing the genome of *S. zooepidemicus* W60 to identify proteins unique to *S. equi* and differences in regulatory pathways. In a related project, Raksha Tiwari, a Ph.D. student, is sequencing the genome of P9, a temperate bacteriophage of *S. equi* that Jon Spanier and I isolated in 1976. Studies of the genomic sequence of *S. equi* have revealed that genes for some virulence factors are phage encoded and that acquisition of bacteriophage was a key event in formation of the more virulent *S. equi* from its *S. zooepidemicus* ancestor. Outcomes of our research on *S. equi* include a PCR test for detection of *S. equi* in clinical samples, several ELISAs for assay of specific antibodies, and development of a genetically labeled avirulent vaccine strain.

Pneumonia secondary to influenza virus infection or to high temperature stress (summer pneumonia) has been shown to be due to invasion of lung by a single clone of *S. zooepidemicus* selected from the indigenous tonsillar flora.

LEPTOSPIROSIS

Abortion and recurrent uveitis are the most familiar clinical manifestations of leptospira infection of horses. The very high levels and multiple specificities of antibodies in sera of recently aborted mares have been valuable tools for detection of leptospira proteins up regulated at body temperature or expressed only during infection. Commercial vaccines prepared from *Leptospira* spp. cultured at 30°C are deficient in these important immunogens, so new generation vaccines are likely to contain one or more of these proteins. My laboratory has characterized three novel host-induced proteins and shared in characterization of a fourth. Useful by-products of these studies have been development of ELISAs to differentiate vaccine from infection responses and improved PCR methodology for detection of leptospira in horse urine by Sergey Artiushin. Finally, Ashutosh Verma, a Ph.D. student in my group, has characterized several novel leptospira proteins uniquely expressed and immunogenic in uveitic eyes. This information will have implications regarding the composition of new generation leptospira vaccines for use in species susceptible to uveitis.

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A Broad Perspective on Chondrocyte Gene Expression

SYNOVIAL JOINTS ARE COMPOSED OF DIFferent tissues that function together to achieve movement with an amazingly low coefficient of friction between apposing bone surfaces. In health, joints move smoothly and painlessly with a full range of motion. Joint injuries, however, can cause inflammation and degenerative changes in multiple tissues, including the joint capsule, synovial membrane, ligaments, articular cartilage, and the bone underneath the articular cartilage. Unfortunately, not all of these tissues have good regenerative properties. Cartilage, in particular, does not heal efficiently. The cells in articular cartilage (called *chondrocytes*) have only a limited capacity to repair structural defects in the joint surface, which is a primary reason why osteoarthritis is a chronic and progressive disease. Lesions in the articular surface do not repair well, and joint function often deteriorates further over time.

Many scientists interested in synovial joints and osteoarthritis study the cell biology of chondrocytes. There are many important questions to investigate. How does the normal function of chondrocytes change as a horse matures? How do healthy chondrocytes

respond when a horse starts into heavy work and the biomechanical stresses placed on joints increase? What variables compromise the function of chondrocytes, allowing structural lesions in articular cartilage to develop? How are chondrocyte functions altered by medications and other therapeutic interventions? Why are chondrocytes normally unable to repair a lesion in the joint surface and fully restore the structural and biomechanical integrity of articular cartilage? If and when structural lesions in the joint surface develop, what can be done to enhance the regenerative potential of chondrocytes?

An important strategy to investigate these and related questions is an analysis of chondrocyte gene expression. The basic premise is that valuable insight about cartilage can be obtained from studying changes in the pattern of chondrocyte gene expression. New technology is becoming available to make this scientific strategy much more powerful and efficient. Traditionally, gene expression has been studied one gene at a time, and scientists have been quite limited in the number of genes they could evaluate in any given experiment. With approximately 30,000 genes in the total genome, this has been an inefficient process. The new methods allow scientists initially to take a much broader perspective, screening expression across large subsets of genes in a single experiment. This capability enables informed decisions to be made subsequently on which individual genes should be most interesting to focus on at greater detail. Essentially, scientists can evaluate the "forest" before making a decision on which individual "trees" should be investigated further. To take advantage of these experimental genomic strategies, we have been working over the past two years to develop a cDNA clone set representing 9,322 different genes expressed by chondrocytes in equine articular cartilage. This clone set will enable gene expression profiling of experimental samples on a broad scale, generating data from thousands of genes not routinely studied in cartilage and more than 1,000 transcripts that do not match any functionally annotated genes. Availability of equine-specific DNA sequences is very important, because it enables our studies to be performed with specificity and sensitivity on samples isolated from horses. For scientific research on equine lameness, expression profiling of chondrocytes holds the promise of identifying both novel genes that are functionally important in cartilage and quantitative changes in gene expression that will provide valuable insight into arthritis and other joint diseases.

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Equine Infectious Anemia (EIA): 25 Years and $600 Million Later

OWNERS IN THE UNITED STATES HAVE PAID over $600 million to test for EIA since 1980. Horse owners and veterinarians in the early 1970s remember the benefits associated with using for the first time an accurate test (AGID, or Coggins test) to identify carriers of EIA virus (EIAV). The risks of acquiring EIA at major racetracks and breeding farms went to zero if control guidelines were followed. In 2003 at a cost of about $50 million, 273 test-positive equids were found in the United States, a rate of 0.015%. We are evaluating current control efforts to improve the benefit/cost ratio. Estimates indicate we can offer the current or higher level of protection against EIA at considerable savings by consolidating present state programs into regional ones. The USDA has been formally requested to initiate a National State-Federal Cooperative Program that will foster such regional efforts.

Why no vaccine? Can we find the remaining reservoirs of infection? When is testing no longer required? Are our diagnostics optimal?

Our research on EIA is directed at these questions. The etiologic agent of EIA, a cousin of HIV, is an equid-specific lentivirus that causes persistent infections in equids and mutates at a high rate. Thus, EIAV poses challenges

to equids that parallel those posed by HIV to humans.

Vaccine design and evaluation are high priorities in our laboratory. Our best candidates show promise; they appear to protect horses against infection and disease when exposed to our pathogenic lab strain of EIAV. Can we produce vaccines that are safe and effective against all strains of EIAV? Our current NIH grant to evaluate diversity of EIAV and its effects on vaccine efficacy addresses that question. We have collaborated with Dr. Ron Montelaro at the University of Pittsburgh on these and other basic questions on EIA since 1978.

Vaccines for EIA and HIV may be elusive because these viruses exploit a fundamental property of the immune system that limits responses to few of the many potential targets (epitopes) present on infectious agents. Although this "immunodominance" is not usually a weakness, mutable pathogens such as EIAV and HIV evade immune surveillance when these immunodominant epitopes change. Other subdominant epitopes may be more highly conserved and might stimulate broader and more effective responses that protect against infection/disease following lentiviral exposure if the immunodominance could be circumvented. The role of immunodominance in EIA is a high priority for Dr. Frank Cook, a molecular virologist on our research team.

Infection with EIAV remains an important cause of morbidity and mortality in equids in many areas of the world, and EIA serves as a valuable animal model for host responses to lentivirus infections. To date, no practical methods have been found to replace the current diagnostics based on detection of antibodies against EIAV in serologic tests in AGID and ELISA test formats. We collaborate with the USDA and diagnostic test kit manufacturers, monitor the licensed kits, and suggest improvements in the routine diagnosis of EIA. We have proposed models to use existing test kits that are the basis for a new three-tier laboratory system being evaluated by the USDA.

We have the tools to eradicate EIA. If we continue to live with the infection, managing the risk will be more cost effective when regional "EIA-free" certifications are adopted.

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Equine Arteritis Virus

SINCE MY APPOINTMENT TO THE FREDERick L. Van Lennep Chair in Equine Veterinary Science in 1988, my primary research interest has focused on aspects of the biology and epidemiology of equine arteritis virus, the causal agent of equine viral arteritis, with particular reference to occurrence of the carrier state.

Earlier studies in this department confirmed that establishment and persistence of equine arteritis virus in the horse is testosterone-dependent, which explains why the carrier state has only been recorded in intact, sexually mature colts or stallions. Based on field investigative studies over the past 20 years, frequency of the carrier state can vary from less than 10% to as high as 70% in naturally infected stallions. Furthermore, not all carrier stallions remain persistently infected for life. It is now widely accepted that the carrier stallion is the principal reservoir of equine arteritis virus in various equine populations worldwide.

There have been authenticated instances where global spread of equine arteritis virus has resulted from the international movement of persistently infected stallions or the shipment of infective semen. It is little wonder, then, that all the major horse breeding countries with the exception of the United States bar the importation of such animals or their semen, regardless of the genetic potential that they may represent. Aside from the significant economic repercussions that can result from restrictions on the international trade in carrier stallions or infective semen, most stallions suffer a major decline in commercial marketability and value domestically if they are confirmed virus carriers.

In view of the significance of the carrier stallion in the epidemiology of equine arteritis virus and the economic consequences arising

therefrom, there is an obvious need to expand upon earlier studies of the carrier state, notwithstanding the availability of a safe and effective vaccine with which to protect against the infection. Current research is focused in several areas—establishment of an *in vitro* model of viral persistence, investigation of host-related factors that play a role in defining whether equine arteritis virus persists in the reproductive tract of certain individual stallions and not others, elucidating the basis for spontaneous clearance of the carrier state in particular stallions, and determining whether viral pathogenicity can be modulated by long-term persistence in the stallion.

Through increasing our understanding of the carrier state, hopefully it will be possible to develop safe and reliable strategies for eliminating the virus without the risk of compromising the future fertility of these stallions. Successful treatment of stallions persistently infected with equine arteritis virus would have the major economic advantage of enhancing the commercial value and marketability of such animals and eliminating any impediment to their export to most countries in the world.

The significant contributions to past and current studies on the carrier state by various individuals over the years, most notably Dr. William McCollum, Professor Emeritus, University of Kentucky, and Drs. James MacLachlan and Udeni Balasuriya, University of California, Davis, is acknowledged with gratitude.

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