



EQUINE DISEASE QUARTERLY

FUNDED BY UNDERWRITERS AT LLOYD'S, LONDON, BROKERS AND THEIR KENTUCKY AGENTS

JULY 2012

Volume 21, Number 3



COMMENTARY

IN THIS ISSUE

Commentary 1

International 2

- Dourine in Italy

National 3

- Equine Laminitis
- Equine Melanoma and the Nature of Malignancy
- *Corynebacterium pseudotuberculosis* Infections in Horses: A Re-emerging Disease in the USA and Canada

Kentucky 6

- Spring 2012 Tornadoes

A RECENT TELEVISION documentary about stem cell fraud in human medicine posed this question: "Are stem cells the 'snake oil' of the 21st century?" The documentary focused on the growing, highly lucrative business of fake stem cell cures that reportedly resulted in a federal investigation. With public awareness of failures in stem cell therapy newly heightened, it is important to reflect on our situation in equine medicine.

Stem cell treatments for horses have been available since at least 2005. They have become a relatively common treatment for many equine musculoskeletal injuries, often promoted as a panacea for bone, cartilage, and tendon healing. Internet testimonials and videos claiming miraculous cures in individual patients with neurologic and laminitic conditions have fueled often irrational exuberance and extravagant expectations. To date, no published controlled clinical studies show efficacy in use of stem cell treatment for any of the conditions being treated. In fact, the two most recent studies evaluating stem cells, one in a tendonitis model and the other in a cartilage defect model, showed no significant difference with their use, either clinically or histologically, as compared to controls in which stems cells were not used.

Papers have touted stem cells' miraculous ability in the laboratory; however, once administered to the patient, the situation changes dramatically. A multitude of poorly understood factors likely mitigate the activity of stem cells once administered. Patients have their own stem cells, and wound healing is modulated through a complex array of molecular events, including cytokine signaling. Equally important, stem cell culturing methods and source tissues (bone marrow, adipose, fetal cells, and cord blood) vary among laboratories.

Are stem cells from any source good for everything? Most scientists would agree this is unlikely, and future treatment will likely involve selection of stem cells with a particular phenotype or genetic modification in order to treat a specific disease. The most basic questions remain. How do we know that the stem cells from a particular laboratory or process are not terminally differentiated fibroblasts that have absolutely no biologic effect? How do we know they will persist in the wound environment long enough to have a biologic effect? How do we know they are of the correct phenotype? Plasticity, differentiation into the desired tissue type, and/or feeder effects may be present in the petri dish; however, mechanisms once injected into the patient have yet to be elucidated.

With a virtual absence of oversight and regulations, equine veterinary use of stem cells has dramatically increased, resulting in over a dozen university, private enterprise, and private practices culturing allogeneic and/or autologous stem cells for use in horses. Expert opinion, testimonials, and anecdotal evidence are not in short supply. When coupled with a lack of good clinical evidence or even a good understanding of potential mechanisms, they threaten to pull stem cell therapies into the abyss often occupied by nutraceuticals and supplements.

Stem cells hold promise. However, clinical acceptance has greatly outpaced the scientific evidence. That evidence needs to be supplied, with both professional organizations and those producing stem cells taking the lead. Finally, practitioners using stem cells also have a responsibility to accurately represent these therapies to their clients.

UK

UNIVERSITY
OF KENTUCKY

College of Agriculture
Department of Veterinary Science

LLOYD'S

**CONTACT: Dr. Wesley Sutter, (859) 967-0770,
wsutter@lexingtonequine.com,
Lexington Equine Surgery & Sports Medicine,
Lexington, Kentucky.**



Dourine in Italy

DOURINE IS A SEXUALLY transmitted parasitic disease of equidae, caused by the flagellate protozoan *Trypanosoma equiperdum*. Recent phylogenetic investigations suggest that *T. equiperdum* and *Trypanosoma evansi*, the agent of surra, are subspecies of *Trypanosoma brucei*, transmitted by tsetse flies and widespread throughout Africa. Dourine is endemic in many areas of Asia, Africa, Russia, parts of the Middle East, South America, and southeastern Europe. In Italy, it was originally eradicated in the 1940s, but in the 1970s a serious epidemic occurred.

Dourine is the only trypanosomiasis transmitted solely by the venereal route. The pathogenicity of different *T. equiperdum* strains is variable.

The diagnosis of dourine can be difficult, as clinical signs and lesions may be absent. Direct diagnosis can also be problematic, given the low number of parasites normally present in infected tissues and the mild, short-lasting parasitemia. Additionally, there is serological cross-reactivity with other trypanosomes.

In May 2011, a stallion undergoing routine testing in Italy for stud purposes tested positive for dourine in the complement fixation test. The ensuing epidemiological investigation revealed four other outbreaks epidemiologically linked with the first (see Table 1, pp. 3-4).

A nationwide serological survey of all officially recognized stallions and females of reproductive age (> 2 years) ordered by the national veterinary authority revealed two new outbreaks in the regions of Campania and Puglia.

Two stallions and four mares exhibiting clinical signs from different outbreaks were transferred to the Istituto G. Caporale in Teramo, Italy, in order to study the pathogenesis of the disease and to carry out further diagnostic evaluation. The main signs observed in these horses were rapid weight loss; labial ptosis; swollen joints; urticarial, plaque-like skin lesions; ventral edema including the scrotum; evidence of lymphatic stagnation; and congestion of the genital mucosa.

Tissues were harvested and tested by a specific real-time PCR assay for the *Trypanozoon* subgenus. The following samples were positive (in some cases also on direct microscopic examination): mammary tissue, secretions and draining lymph nodes, plaque-like skin lesions, popliteal lymph nodes, cerebrospinal fluid, clitoral groove smear, urine and tear secretion, and intra-articular fluid. *T. equiperdum* was isolated from the mammary secretion of a naturally infected mare inoculated in the scrotum of a male rabbit.

The distribution of the premises involved, prevalence on the premises and in the surrounding area, and the animals that tested positive (all adult animals used for reproduction) do not correlate with a disease transmitted mechanically by insects like surra. On the contrary, these factors are congruent with a disease transmitted by coitus, like dourine. Also, five of the seven outbreaks were linked by the movement of breeding animals. To date, healthy horses living in contact with symptomatic and parasitemic horses during periods when vectors of *T. evansi* are likely to have been active have neither developed antibody nor signs of surra.

Currently in progress are studies of the pathogenesis of dourine; molecular characterization of the strain isolated; comparative serologic testing including Western blot, ELISA, and immunohistochemistry; and attempts at *in vitro* cultivation of the *T. equiperdum* isolate and its transmission *in vivo*.

CONTACT: Dr. Rossella Lelli, (39) 0861-332204, r.elli@izs.it; Dr. Massimo Scacchia, (39) 0861-332204, m.scacchia@izs.it; Dr. Vincenzo Caporale, (33) 144-151888, v.caporale@oie.int; Istituto G. Caporale, 64100 Teramo, Italy.



Equine Disease Quarterly

Editors

Roberta Dwyer
Peter Timoney
Neil Williams

Staff

Diane Furry
Martha Jackson
Linda Millercox

Correspondence should be addressed to the editors, Department of Veterinary Science, Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, Kentucky USA, 40546-0099
Telephone (859) 257-4757
Fax (859) 257-8542

Internet address:
<http://www.ca.uky.edu/gluck/index.htm>

Material published in the Quarterly is not subject to copyright. Permission is therefore granted to reproduce articles, although acknowledgment of the source and author is requested.

The University of Kentucky is an Equal Opportunity Organization.



Printed on recycled paper

The First Quarter Report for 2012 from the International Collating Centre was not available at press time.



NATIONAL

Equine Laminitis

EQUINE LAMINITIS CAN BE a devastating result of many different disease processes in the horse, including, most commonly, sepsis and endocrinopathies. The two primary types of endocrinopathic laminitis are equine metabolic syndrome (seen most often in the obese horse), and Cushing's syndrome in older horses (characterized by high levels of circulating steroids produced by a pituitary tumor). Interestingly, the "target" tissue in the horse for sepsis, equine metabolic syndrome, and Cushing's syndrome is the digital laminae.

Most likely the main reason the digital laminae are the primary target is because no other soft tissue structure in species injury/dysfunction will result in the entire collapse of the musculoskeletal system of the animal. The laminae basal epithelial cells are exposed to incredible forces (supporting the entire weight of the horse).

Septic conditions in the horse that can lead to laminitis include gastrointestinal disease (surgical lesions, diarrhea/enteritis from infectious agents, or carbohydrate overload), retained placenta in the post-foaling broodmare leading to a uterine infection, pleuropneumonia, and any other infection in which enough tissue is compromised to result in systemic effects. In most of these cases, toxins absorbed from Gram-negative bacteria are thought to be responsible for the systemic problems such as laminitis. However, bacterial infections from other types of organisms can also result in laminitis. Most progress has been made in studying sepsis-related laminitis, as most experimental models for laminitis mimic this condition. Systemic inflammation leading to inflammatory injury to the laminae tissue has been reported in sepsis-related laminitis in horses. In the laminae, this injury is characterized by adhesion and migration of circulating white blood cells out of the blood vessels into the laminae tissue. This is accompanied by massive increases in expression of inflammatory proteins such as cytokines (a 10-fold to > 2,000 fold increase in expression) and cyclooxygenase-2 (COX-2, the enzyme which is targeted by non-steroidal anti-inflammatory drugs such as phenylbutazone or flunixin). These events most likely cause injury to the laminae basal epithelial cells, leading to disruption of their critical cellular events, including adhesion

to the underlying matrix. The matrix itself may also be injured by the release of matrix-degrading enzymes by leukocytes, epithelial cells, and other cell types in the laminae.

Equine metabolic syndrome (EMS), which includes pasture-associated laminitis, is now the most common type of laminitis reported by veterinarians. Although the animals affected are commonly obese, animals in "show shape" that are not overtly obese also succumb to EMS-related laminitis. A consistent factor in the horse or pony with EMS is insulin resistance, with the animals usually exhibiting increased circulating insulin concentrations. It has been suspected that laminae injury in EMS was from an inflammatory event as discovered in sepsis-related laminitis. However, recently presented data indicate that the high circulating insulin concentration itself can induce laminitis, with limited evidence of inflammation in the laminae.

The other type of endocrinopathic laminitis, Equine Cushing Syndrome (ECS), may have a pathophysiologic mechanism similar to that of EMS, as ECS horses similarly have high levels of circulating insulin. However, it is possible that the glucocorticoids (GCs) may be playing a role in disruption of the cell biology of the laminae keratinocytes in ECS.

The pathophysiology of supporting limb laminitis, the type suffered by Barbaro, is the type of laminitis about which we presently have the least knowledge. With this type, excessive weight bearing (usually due to a painful injury on the opposite limb) results in laminae failure. The recent interest supporting limb laminitis has resulted in several studies being funded by equine foundations. Hopefully, these studies will further elucidate the pathologic mechanisms (and thus therapeutic targets) for this equally devastating form of laminitis. Thus, laminitis is likely the end product of a diverse array of disease processes that lead to disruption and failure of a highly evolved cell type that is exquisitely sensitive to injury—the laminae basal epithelial cell.

CONTACT: Dr. James Belknap, (614) 292-6661, james.belknap@cvm.osu.edu, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio.

Equine Melanoma and the Nature of Malignancy

WHETHER EQUINE MELANOMA is a benign or malignant neoplasm has been discussed and debated for at least 100 years. Many equine practitioners, by virtue of their training and experience, consider it a common benign growth of the skin, particularly of gray horses, which is precisely as stated in a manual on recognition and treatment of equine diseases published by the United States Department of Agriculture in 1916. However, equine melanoma was considered at that time a serious malignancy—“melanosarkoma”—in thought and German literature (1909).

During pathology training, many veterinarians are taught that most equine melanomas are focal aggregates of locally-growing, variably pigmented cells—akin to human moles (pigmented nevi), which is a somewhat classic view of benign tumors. Armed with this knowledge, they counsel owners of horses with tumors that melanoma is slow-growing and of little consequence. This theme is common in pre-purchase examinations. The statement “your horse will die of something else” is arguably correct considering that the truly common causes of death of horses include musculoskeletal injuries (and euthanasia), gastrointestinal catastrophes (and euthanasia), and a variety of cardiorespiratory lesions resulting in poor performance (and euthanasia).

Little justification exists for prolonging discussion of the nature of equine melanomas. Equine melanoma is a progressive malignancy.

Like all neoplasms, equine melanoma must begin with the transformation by mutation of a single stable cell faithfully reproducing its genotype and phenotype to that of a neoplastic cell displaying a propensity for unregulated growth and variations in differentiated phenotype. This process is not in dispute. The small melanoma under the tail of the gray horse already has formed a cluster of cells that infiltrate and compress surrounding skin and connective tissue—two things that normal, differentiated melanocytes do not do. These small melanomas may be quiescent for many years.

In 30-40% of affected horses, these cell clusters continue to evolve at one or more sites. We know from the study of many animal and human tumors (malignant gliomas, for example) that the

progressive growth of, and invasion by, tumors is driven by further mutation of the unstable neoplastic genome. Progression involves the selection of neoplastic cells capable of exploiting their environment—acquiring and processing nutrients, soliciting vascular growth, avoiding detection and destruction by immune and inflammatory systems—and increasing their numbers. As they progress, these neoplastic cells become more and more difficult to control and eradicate, fully expressing their malignant character.

It is common to see severely affected horses. A small cutaneous lesion that has been present for months to years begins to grow rapidly and may ulcerate. In some cases, multiple masses form at different sites, possibly representing simultaneous growth of several unique tumors (something being investigated with genotyping), rather than metastatic spread. Virtually all of these tumors are infiltrating tissues around them, a well-recognized problem in Stage III-IV human melanoma (in which the survival rate is less than 10% five years after diagnosis). These malignancies in horses are difficult to excise and are rarely removed completely. When they interfere with defecation, reproduction, or eating, they cause suffering and result in death (euthanasia).

Equine melanomas are not benign and should not be ignored. The current proven method for control of these tumors is removal, through surgery, by laser, or with cautery. Veterinarians should recognize the need for this approach and deal realistically with horses with equine melanomas until we have a better understanding of the mutations that drive their formation and progression.

CONTACT: Dr. John L. Robertson, (540) 231-4643, drbob@vt.edu, Department of Biomedical Sciences & Pathology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, Virginia.

***Corynebacterium pseudotuberculosis* Infections in Horses: A Re-emerging Disease in the USA and Canada**

INFECTION IN HORSES caused by the Gram-positive bacterium *Corynebacterium pseudotuberculosis* can assume many forms. Deep intramuscular abscesses in horses caused by *C. pseudotuberculosis* were first reported in San Mateo County, California, USA in 1915. Since that time, the disease commonly referred to as “pigeon fever” can be considered one of the most frequent infectious diseases in the western USA. Infections tend to occur as sporadic cases on a farm or as outbreaks involving hundreds of horses in a region. Disease incidence is increasing, possibly in association with climate change. Unprecedented epidemics in the past decade have affected tens of thousands of horses in Texas, Louisiana, Colorado, New Mexico, Utah, Wyoming, Kentucky, Missouri, Oregon, Washington, Idaho, and British Columbia, Canada, involving regions historically low in prevalence. High environmental temperatures and drought conditions preceded all reported outbreaks of this soil-dwelling organism. The most common clinical form of the disease is characterized by external abscesses in the pectoral or ventral abdomen, hence the term “pigeon fever” due to the swelling of the horse’s pectoral region resembling a pigeon’s breast. Two other clinical forms of the disease include internal organ involvement such as liver, lung, kidney, or spleen abscesses and infection of the limbs, termed “ulcerative lymphangitis,” which appears as a severe cellulitis with multiple draining ulcerative lesions.

The portal of entry for the bacteria is through abrasions or wounds in the skin or mucous membranes. Insects such as *Haematobia irritans*, *Musca domestica*, and *Stomoxys calcitrans* can act as mechanical vectors of this disease. The regional location of abscesses suggests that ventral midline dermatitis is a predisposing cause of infection. Due to the variable incubation period, which is typically three to four weeks or longer, ventral midline dermatitis may not be present at the time of maturation of the abscesses.

Antimicrobials are indicated for horses with ulcerative lymphangitis and for horses with internal abscesses. The use of antimicrobials for external abscesses is not necessary in most horses. Antimicrobial therapy may be justified when signs of systemic illness are present, such as fever, depression, and anorexia, and when extensive cellulitis or lameness is present.

Corynebacterium pseudotuberculosis is susceptible *in vitro* to most commonly used antibiotics in horses. The average duration of antimicrobial therapy for internal infection is four to six weeks and is best determined by repeat abdominal ultrasound and clinicopathologic results. Horses with ulcerative lymphangitis or cellulitis should be treated early with antimicrobials, or residual lameness or limb swelling may occur. Until a protective bacterin or toxoid is developed for horses, we can only suggest that horse owners practice good sanitation and fly control and avoid unnecessary environmental contamination from diseased horses. Presently there is no evidence that diseased horses within a stable should be quarantined other than by paying strict attention to insect control. The feed-through products containing cyromazine are safer than organophosphate products and may reduce the incidence of disease by controlling vector populations. Fastidious wound care and prevention of ventral midline dermatitis is also important in preventing infection from a contaminated environment.

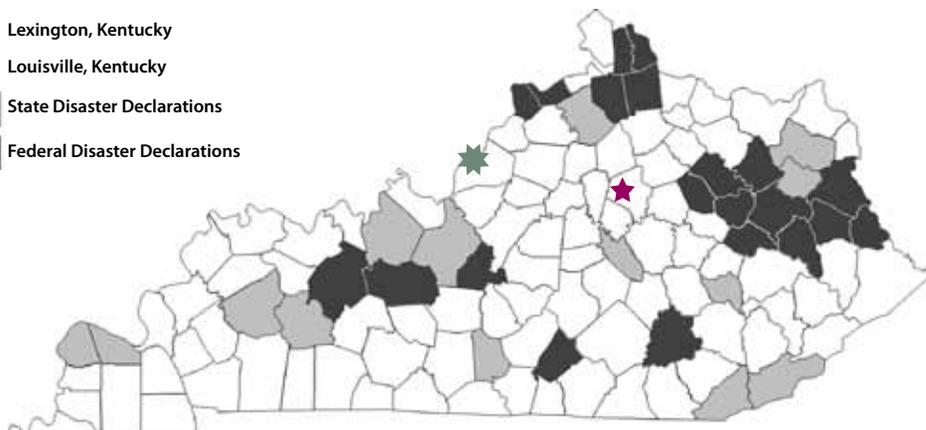
**CONTACT: Dr. Sharon Spier, (530) 752-0292,
sjspier@ucdavis.edu, School of Veterinary Medicine,
University of California, Davis, California.**



KENTUCKY

Spring 2012 Tornadoes

- ★ Lexington, Kentucky
- ★ Louisville, Kentucky
- State Disaster Declarations
- Federal Disaster Declarations



On February 29 and March 3, 2012, tornadoes swept across Kentucky, resulting in 35 counties with state disaster declarations, of which 21 were subsequently declared federal disaster counties. Agricultural damage included injured and dead livestock, flattened barns and fences, and pastures contaminated with debris. Coordinated efforts among many organizations, including the University of Kentucky Cooperative Extension Service, enabled farmers to obtain critically needed assistance.

Andrea Husband Higdon (859) 257-7868, andrea.husband@uky.edu, Cooperative Extension Service, University of Kentucky, Lexington, Ky.

Equine Disease Quarterly Newsletter

Department of Veterinary Science
 Maxwell H. Gluck Equine Research Center
 University of Kentucky
 Lexington, Kentucky 40546-0099

Address Service Requested

Table 1.
**Epidemiologically linked outbreaks
of dourine in Italy.**

			EQUIDS			
	Region	Province	Examined	Positive	With Clinical Signs	
O U T B R E A K	1	SICILY	<i>Catania</i>	27	1	1
	2	SICILY	<i>Catania</i>	5	1	0
	3	CAMPANIA	<i>Naples</i>	8	2	0
	4	CAMPANIA	<i>Naples</i>	3	1	0
	5	CAMPANIA	<i>Naples</i>	17	5	5
TOTAL			60	10	6	