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IN THIS ISSUE

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LLOYD'S

Commentary

With the recent events of the past year, public attention has focused on bacterial infection as an instrument of terror. The terms "spore" and "toxin" have been widely discussed and become part of public lexicon. At the time of writing, the disease anthrax occupies a significant portion of personal conversation and news-related programs in the media. The general public is now versed in how anthrax is spread, its toxin-related pathogenesis, and the appropriate treatment. Knowledge that bacteria can cause harm by the production of powerful toxins is also becoming mainstream.

Another potential malicious use of bacteria is through the mass production of toxin with dissemination of the toxin, rather than the bacteria itself. Such a candidate is the exceedingly lethal toxin of *Clostridium botulinum* (botulism toxin). In this issue of the *Quarterly*, an important disease of foals caused by another species of clostridium, *Clostridium perfringens*, is discussed.

Veterinary medicine has long dealt with the scourge of anthrax. Virtually all warm-blooded animals are susceptible (horses are very susceptible, usually dying 2-3 days after signs begin) and there are known areas in the United States where anthrax infections tend to occur on a regular basis. A number of other equine diseases are the result of toxin production by bacteria, and horses and man are among the most susceptible species to the effects of bacterial toxins.

In general, bacteria cause disease either by invasion and multiplication in host tissues, damaging host cells, or by the elaboration of toxins. Bacterial toxins are divided into two main groups: endotoxins derived from the cell wall of gram-negative bacteria, and exotoxins produced inside gram-positive bacteria.

Exotoxins are potent toxins that may be secreted through the cell wall or released upon lysis of the bacteria. They have specific effects depending on the source and type of exotoxin. Exotoxins can destroy other cells (cytotoxins), affect nerve cells as in botulism and tetanus (neurotoxins), act in an enzymatic fashion to destroy tissue (α-toxin of *Clostridium perfringens*), or, in the case of lethal toxin from anthrax, induce systemic shock and death through the stimulation of macrophage cytokine production.

Endotoxin, a structural component of the cell wall of gram-negative bacteria such as *E. coli* and *Salmonella*, is released from the bacteria upon death or during multiplication. Endotoxin, unlike exotoxin, is not directly toxic to cells and tissues but rather exerts its profound effects via stimulation of the body's immune system, causing release of substances that give rise to inflammation. When sufficient amounts of endotoxin reach the bloodstream, systemic responses occur that can lead to a condition called endotoxemia (or septic shock if severe). Signs of endotoxemia include depression, fever, hypotension, inappropriate activation of the coagulation system, and shock.

The fact that the body's response to endotoxin can ultimately lead to organ failure and death is perplexing in that processes designed to protect us from bacterial infection can, if excessive, lead to our demise. This paradox is leading researchers to design new therapeutic approaches to suppress or counteract aspects of the body's reaction to bacterial toxins, in addition to conventional approaches that directly target the bacteria. ■

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International

Third Quarter 2001

The International Collating Centre, Newmarket reported the following disease outbreaks.

The respiratory and abortion forms of equine herpes virus (EHV) infection were reported in both Thoroughbred and non-Thoroughbred horses in France. In the United Kingdom a single case of abortion attributable to EHV-4 was diagnosed. Loss of performance among a group of horses in the United Kingdom was attributed to EHV infection based on serological evidence. A fatal case of Equine Protozoal Myeloencephalitis (EPM) was diagnosed in Japan during July in a horse imported from the United States during March 2000.

Influenza was widely reported in France and on a single premise in the United Kingdom, the latter among unvaccinated animals. Serological evidence of piroplasmosis caused by *Babesia equi* and *caballi* was reported from Switzerland in nine animals. *Pneumocystis carinii* was diagnosed as causing the death of three foals from interstitial pneumonia in Turkey. France reported that it is now free of rabies. Strangles was reported from three states in Australia, New South Wales, Queensland and Victoria; in Ireland on 10 premises; and from Italy, Sweden, Switzerland, and the United Kingdom. ■

National

West Nile Virus Update

As of November 20, evidence of West Nile Virus (WNV) has been reported extensively in eastern North America extending from Maine and Ontario in the north to Florida in the south, as illustrated in **Figure 1**. Fifty-three human cases, 5 fatal, have been confirmed in 9 states of the United States and 1 case in the Cayman Islands. The median age of all cases is 70 years, ranging from 36 to 90.

Four hundred and twenty six equine cases, with a mortality rate of 25%, have been confirmed in 19 states, the vast majority in Florida. Among the wild-bird population, there have been 4,604 cases in crows and 1,497 in other birds distributed over 27 states and the District of Columbia. The number of positive mosquito pools identified in 15 states and the District of Columbia is 756.

Updated information can be obtained at www.aphis.usda.gov/oa/wnv/index.html. ■

Reproductive Success in Broodmares— Part 2

Part 1 of "Reproductive Success in Broodmares" (*Equine Disease Quarterly* 9[3] April 2001) discusses differences between *per-season* and *per-cycle* success rates using breeding data for the Thoroughbred *population* (data gathered over five years) and four central Kentucky Thoroughbred *farms* (data gathered over two years). **Figure 2** summarizes this information.

This article discusses factors contributing to reproductive performance of the population and mares.

Figure 2.
Data Included in the Study

	Population	Farms
Number of stallions	40,512	96
Number of mares	408,275	4,775
Number of live foals	241,958	3,442
Foaling rate *per-season*	59%	72%
Foaling rate *per-cycle*		42%

Effect of Booksize

Booksize, or size of a stallion's book, is defined as the number of mares bred to a stallion during one season. As shown in **Figure 3**, the percentage of mares represented in each booksize is highly skewed toward the smaller books for the population mares, whereas it is centered around 50-60 mares per stallion for the farms. For both groups, the proportion of mares delivering live foals rose as booksize increases **(Figure 4).** This is likely associated with improved man-

agement as booksize increases.

The stallions with the larger books are well known, are on larger farms, and likely represent a greater financial investment in stud fees for the mare owners. Therefore, it is likely that these stallions and the mares bred to them were subjected to greater management intensity than for horses in the overall population. For example, the managers of mares being sent to these stallions may have found it financially feasible to utilize lights to hasten the first ovulation of the year, frequent teasing schedules, early pregnancy testing by ultrasonography, and other management practices to ensure an optimal chance of producing a live foal.

In the overall population, the foaling rate for mares in smaller books was about 50% *per season*. The 70% live foaling rate for mares bred to stallions with booksizes of 75-100 is about what would be expected if the *per-cycle* foaling rate were 50% *and* the mares were bred on average two cycles.

Effect of Mare Availability

A major factor in the *per-season* reproductive success of a mare is the potential number of cycles on which she can be bred—or on which she is *available* for breeding. For a given breeding season, the best indicator that a mare is available for breeding is the fact that she is indeed sent to the stallion. The effect of mare availability on the *per-season* live foaling percentage is shown in **Figures 5** and **6.**

For the population, about 68% of mares first bred in February produced a live foal from a cover that occurred sometime during that breeding season, whereas only about 40% of mares first bred in June produced live foals. The live foaling rate for farm mares first bred in February was 82%. The live foaling rate decreased an average of 9% *for each month* that first breeding was delayed.

There are 3 possibilities to explain these results: a decline in fertility as the breeding season progresses, the fact that groups of mares bred earlier in the year are more fertile than groups bred later in the year, and/or more breeding opportunities for the mares bred earlier in the year. While all three factors may play a role, our studies indicate that the third factor has the greatest influence on a successful breeding season.

As can be seen from the graphs, the live foaling rate for mares first bred in February through June was greater for the farm mares than for mares in the overall population, and, in general, farm mares were first bred earlier in the season than other mares in the population.

Figure 1.

West Nile Virus 20

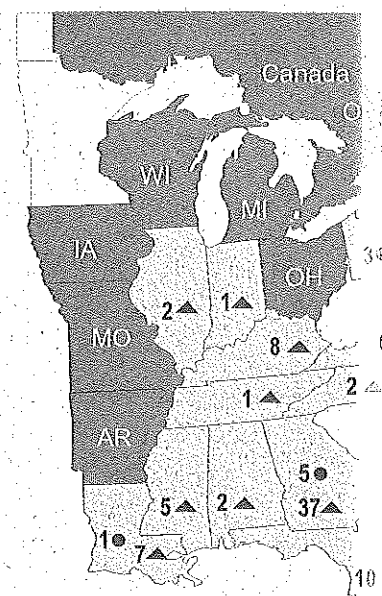


Figure 3.

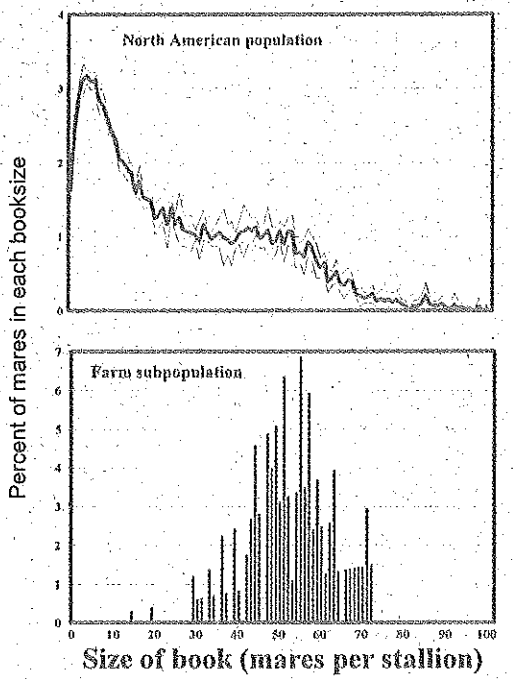


Figure 4.

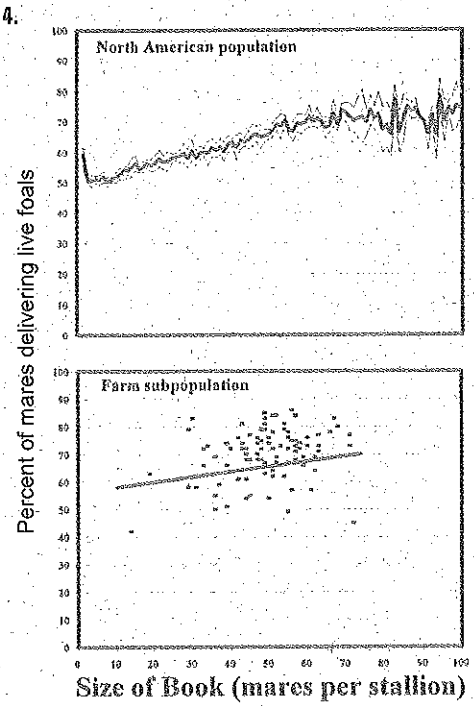
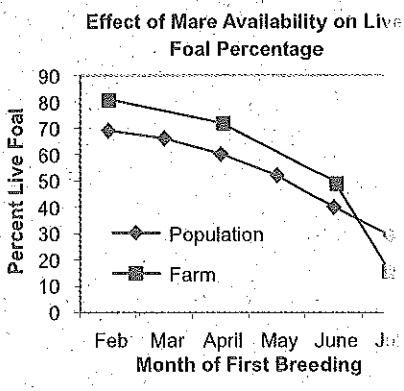
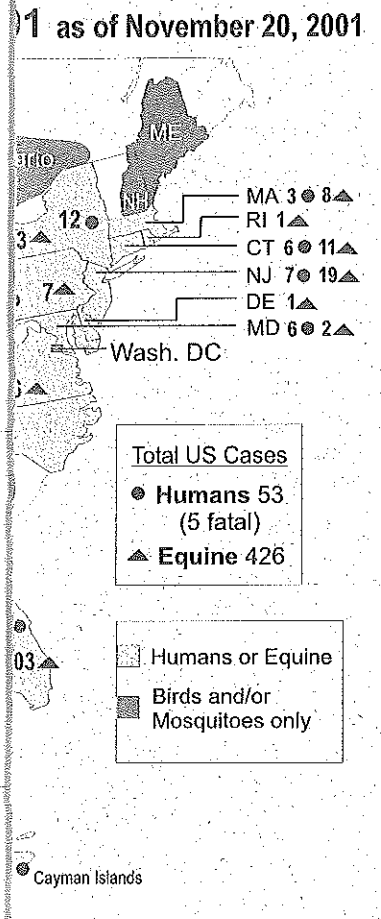


Figure 5.





It is easy to see how these two factors together contribute to the farms having an overall higher success rate than the population.

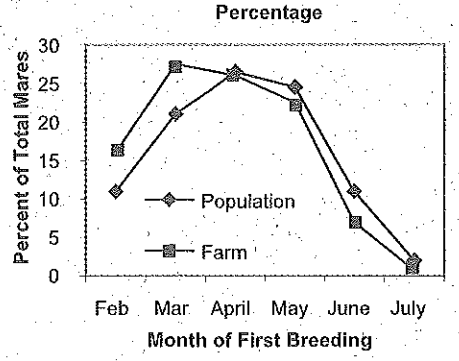
Summary

There are 2 ways to increase the reproductive success of your farm: Increase the *per-cycle* live foaling rate, and increase the *per-season* live foaling rate. The overall goal may not be just to produce more foals, but to produce the high-quality, highly desirable foals more efficiently.

The best way to increase the reproductive success rate *per cycle* is to breed young mares to fertile stallions. Our research at the University of Kentucky is focused on increasing our understanding of conceptus/embryo development in horses, and how the conceptus interacts with the uterine environment. Through this knowledge we should be able to decrease the rate of conceptus loss and hence increase the *per-cycle* reproductive efficiency.

Every manager can take positive steps to increase the *per-season* success rate for his or her farm. The best way to increase the *per-season* success rate is to increase the number of cycles for which mares may be available for breeding during the season. These management practices include: putting mares under lights to hasten the onset of the breeding season, breeding mares at the earliest possible opportunity, and examining them for pregnancy as early as possible after mating/ovulation. If a mare is found not to be pregnant, re-breed as soon as possible, and re-examine pregnancies periodically; if a pregnancy is lost, re-breed the mare during the same breeding season. ■

Figure 6.



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Clostridial Enterocolitis in Horses

Clostridium perfringens has been implicated as a cause of toxin-induced acute gastrointestinal disease in horses. The disease typically affects foals less than 10 days of age and is characterized by abdominal pain, diarrhea (often bloody), toxemia, shock, or death without prior signs. The occurrence is sporadic, with one to multiple horses affected on a farm. The mortality rate is high, with most foals dying within 24 hours of the onset of clinical signs.

The University of Kentucky Livestock Disease Diagnostic Center receives multiple cases of *Clostridium perfringens* enterocolitis yearly. In 1999 there were three confirmed cases, eight in 2000, and six in 2001. All cases (except one 7-week-old foal) were neonatal foals ranging in age from 1-14 days old, with an average age of 3.2 days. The foals were either found dead or died after a short course of illness characterized by depression, toxemia, and diarrhea.

Lesions at necropsy consisted of dehydration, blanching or congestion of the intestinal tract, hemorrhages, and bloody intestinal content. Microscopically, there was necrosis of the mucosal epithelial cells of the small intestine and colon with light inflammatory cell infiltrates and numerous bacteria consistent with clostridia.

The diagnosis of *Clostridium perfringens* enterocolitis was made on the basis of the pathologic findings and culturing of *C. perfringens* from the intestine in high numbers. PCR analysis of 15 of the *C. perfringens* isolates revealed that 9 (60%) were genotype A and 6 (40%) were genotype C. Definitive diagnosis of *Clostridium perfringens* enterocolitis requires demonstration of toxins in the intestine or feces of the affected foal. This is difficult in animals dying from this disease due to rapid inactivation of toxin and overgrowth of other intestinal bacteria during the postmortem period.

Clostridium perfringens is considered part of the normal flora of the intestine of horses and also an environmental inhabitant. The factors that lead to the development of disease are not clear, but it is believed that there is an alteration

of the normal flora that allows overgrowth of the clostridia. Proposed causes of this include diet changes, antibiotic therapy, stress, or concurrent infection. Other factors that may play a role in the development of clostridial infection are host factors such as age, immunity, and the presence or absence of intestinal receptors for the perfringens toxins. It is the production of potent exotoxins and enterotoxin by *C. perfringens* that causes illness. One of the major toxins produced by *Clostridium perfringens* is broken down by intestinal trypsin. Trypsin is produced by older animals, but is deficient in neonates, which helps to explain the predilection for young foals. Studies have shown adequate serum IgG levels in affected foals, indicating that failure of passive transfer of immunity from colostrum is not involved in development of this disease.

Clostridium perfringens is divided into five types based on toxin production (**Figure 7**). Disease in foals is associated with either type A or C. Enterotoxin can be produced by all types of *Clostridium perfringens* but is most commonly associated with type A. Enterotoxin causes pore formation in cells leading ultimately to cell lysis. The beta exotoxin produced by *Clostridium perfringens* type C is a highly necrotizing and lethal toxin that causes the severe intestinal damage that is characteristic of type C infection. It is commonly type C infected foals that die rapidly with bloody diarrhea and hemorrhagic intestines.

Clostridium perfringens type A is commonly found as normal flora in foals and infection with this type is often less severe due to the absence of beta toxin production. Outbreaks of type A infection in foals can occur with significant morbidity; however, there usually is good response to treatment and low mortality. ■

Figure 7.
Types of *Clostridium Perfringens* Based on Exotoxin Production

Type	Toxin Produced
A	alpha
B	alpha, beta, epsilon
C	alpha, beta
D	alpha, epsilon
E	alpha, iota

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S. Neurona Antibody Prevalence in Horses at Morehead State

Equine Protozoal Myeloencephalitis (EPM) is an infectious, degenerative protozoal disease of the central nervous system of the horse. The causative agent of EPM has been identified as *Sarcocystis neurona*. Dr. David Granstrom developed the first pre-mortem test for the presence of *S. neurona* antibodies in 1993, using a western blot immunoassay. This test is currently the most reliable test that can be performed on serum and cerebrospinal fluid to detect the presence of antibodies to *S. neurona*.

The incidence of *S. neurona* is highest in the eastern United States because of the geographic habitat of the opossum, which is the definitive host. When ingested in high enough levels, the parasite can cause a broad range of clinical signs, including weight loss, muscle atrophy of the rear limbs, incoordination, urinary and fecal incontinence, gait abnormalities, paralysis, seizures, and death.

The purpose of this research was to establish a base-line antibody level for the Morehead State University equine herd in Morehead, Kentucky. The study involved 39 adult horses (stallions, mares, and geldings) of various breeds, ranging in age from 4 years to 33 years of age. All horses were housed at the Derrickson Agricultural Complex on the Morehead State Farm and were housed and fed in a similar fashion.

In this study, one jugular venipuncture was performed on each horse to obtain a sample of whole blood. Each sample was analyzed at Equine Bio-Diagnostics Laboratories, Lexington, Kentucky by western blot to detect the prevalence of *S. neurona* antibody in this group. The results of the study demonstrate a high rate of exposure to *S. neurona* in the herd (32/39) or 82% of the horses sampled (positive, low positive, or weak positive). Forty-four percent (17/39) of horses showed a positive reaction, 20% (8/39) a low positive reaction, and 18% (7/39) a weak positive reaction. There were only 7 antibody negative horses in the MSU herd. The exposure rate for positive and low positive horses was 64% (25/39).

This rate is similar to previously published reports in the veterinary literature of the

seroprevalence of *S. neurona* in central Pennsylvania. The high exposure rate of the horses at Morehead State University is indicative of the large reservoir of *S. neurona* that can be found in the opossum population in Eastern Kentucky. By obtaining this baseline antibody data, this group of horses can now be used for vaccine trials in the future, with less confusion associated with antibody titers from immunization versus titers from actual infection or exposure. The information obtained will be beneficial to horse owners in the Eastern Kentucky region, who may wish to be more informed about the actual incidence of EPM in our area. ■

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Updated Information on New Bibliographies

The Morris Library now has available two new bibliographies: *Laminitis in the horse (1990-2001)* and *COPD [chronic obstructive pulmonary disease] in the horse (1990-2001)*. Both are available in print as well as at the Library's Web site, www.uky.edu/Agriculture/VetScience/morris.htm.

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