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IN THIS ISSUE

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LLOYD'S

COMMENTARY

The unexpected finding of a high frequency of susceptibility to antibiotics of salmonellas from equine specimens for the period June 1994 to June 1996 in central Kentucky as reported in this issue of the *Quarterly* appears to be contrary to trends reported elsewhere in the world. Numerous studies over the past three decades have revealed a high frequency of multiple antibiotic resistance in salmonellas from hospitalized horses. Antibiotic resistant bacteria including *Salmonella* have an obvious survival advantage in environments such as hospitals where there is heavy selection pressure from antibiotic usage.

What is the explanation for the relative absence of resistance among salmonellas from equine cases in central Kentucky, since it is likely that some or most of these cases received antibiotic therapy in the days prior to death? Most were individual cases from different farms suggesting that the salmonella isolates were sporadic individual acquisitions from the environment including feed and wildlife such as birds and rodents. Salmonellas from these sources are usually sensitive to antibiotics. Antibiotic therapy of the affected horses may have been of insufficient duration or have been administered by a non-oral route that minimized pressure for resistance transfer among the intestinal bacteria.

Much remains to be learned and understood about the forces that favor emergence and subsequent decline of antibiotic resistant salmonella clones. It is known that antibiotic resistance in salmonellas may be unstable especially when encoded on large plasmids (DNA containing genetic elements that are separate from the chromosome). Plasmid synthesis imposes a metabolic burden on the salmonella that places it at a major competitive disadvantage in the absence of antibiotic selection pressure. Changes in resistance pattern and serotype may also reflect the changing status in infection of wildlife and feed contamination. Clearly, an ever increasing pool of antibiotic resistant salmonellas is not predestined and inevitable. However, this conclusion is not grounds for complacency.

Antibiotic therapy in cases of equine salmonellosis has to be approached with great caution, with knowledge of the probable antibiotic sensitivity of the salmonella involved, and with the goals only of preventing and controlling systemic invasion. Antibiotic therapy that results in disruption of normal intestinal flora will create a double jeopardy of selection for salmonella clones that have received resistance genes in the intestine and of destruction of the normal bacterial flora that provides colonization resistance to salmonella invasion.

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INTERNATIONAL

Third Quarter 1997

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

Influenza was reported from France, Sweden, United Kingdom and United States. The latter included an outbreak among Thoroughbred racehorses in Puerto Rico during June.

Eastern Equine Encephalitis (EEE) was reported in the United States causing mortality among horses in Alabama, Georgia, Kentucky and Texas during the summer months.

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Two Standardbred stallions in the United Kingdom were reported as being suspected of shedding equine arteritis virus in their semen.

Strangles as usual was the most widely reported disease, from Australia, Denmark, Ireland, Sweden, United Arab Emirates and the United Kingdom. In several countries cases were reported on several premises.

The outbreak of Vesicular Stomatitis continued through the fall in the western United States. As of November 28, 380 cases had been confirmed on premises in Arizona (2), Colorado (272), New Mexico (68) and Utah (38) with a significant drop in the number of cases reported during November.

N A T I O N A L

Oxytocin and Maternal Recognition Of Pregnancy in Mares

It is well established that early conceptus loss (the embryo and its associated extra-embryonic membranes) is a major source of reproductive inefficiency in domestic animals. Early pregnancy in all mammals is characterized by a high fertilization rate followed by a high conceptus loss rate.

In horses, approximately 50% of all pregnancies are lost between fertilization and foaling. About 30% of this loss occurs during the first two weeks of gestation, and the remainder is relatively evenly distributed over the rest of gestation. The actual loss rates on any given farm will vary with such factors as mare age and breeding stallion, as well as numerous on-farm management factors.

Before we can adequately recognize pregnancies at risk of being lost, we must first further our understanding of how the conceptus interacts with the maternal environment, particularly during the first few critical weeks of gestation.

Events that occur between approximately 11 and 15 days of gestation are of critical importance for the establishment and maintenance of a successful pregnancy in horses. The series of events, discussed below, has been termed "Maternal Recognition of Pregnancy." When the mare ovulates an oocyte, the cells in the wall of the follicle (the structure on the ovary that previously housed the oocyte), undergo a rapid series of changes and form a corpus luteum (CL). The CL begins secreting progesterone. The CL secretes progesterone for about 2 weeks, whether or not mating occurred and whether or not pregnancy occurred.

After about 14 days, the mare must "decide" whether to maintain progesterone production or not in response to the biological question: "is there a viable conceptus in the uterus or not." If the answer is "no," the uterus produces prostaglandin $F_{2\alpha}$ (PGF), which ultimately causes the regression of the CL and a decline in progesterone production. The declining progesterone allows for another follicle to develop, another estrous cycle to occur and, ultimately, another opportunity to establish pregnancy.

If the answer is "yes," the conceptus produces an hormonal signal that suppresses uterine PGF production. When PGF production by the uterus is suppressed, then the CL can continue to secrete progesterone to support the growth and development of the conceptus.

If PGF is produced inappropriately during pregnancy, or if the conceptus is not able to suppress it sufficiently, then the pregnancy will be lost. Conversely, if PGF is not produced in the right amounts and the right time at the end of the estrous cycle, then the CL is maintained inappropriately, there is a delay to the next ovulation, and time is lost before the mare can be bred again.

Although we understand this general scenario, as yet we do not have a very good understanding of the specific mechanisms regulating the production of PGF (for luteal regression) or suppression of PGF (for pregnancy maintenance). In sheep and cattle, at the end of the estrous cycle, the CL produces the hormone oxytocin. Oxytocin then stimulates the uterus to produce PGF, and uterine PGF in turn, stimulates the CL to produce more oxytocin. This "positive feedback" between luteal oxytocin and uterine PGF ultimately causes the regression of the CL.

Studies in sheep have shown that, if oxytocin production from the CL is blocked, then luteal regression is delayed and there is a subsequent delay in the amount of time it takes for the ewes to return to estrus. In addition, it is believed that the sheep or cow conceptus is able to uncouple the positive feedback

loop between luteal oxytocin and uterine PGF in order for maternal recognition of pregnancy to occur.

The CL of the mare produces little, if any, oxytocin. The pituitary gland of the mare, like that of other animals, secretes oxytocin, and uterine PGF appears to stimulate pituitary release of oxytocin in mares. However, there is little evidence to suggest that pituitary oxytocin and uterine PGF form a positive feedback loop analogous to that of luteal oxytocin and uterine PGF in ruminants.

If oxytocin is given to mares towards the expected end of the estrous cycle, it will cause the uterus to release PGF. If the oxytocin is given at an equivalent time during pregnancy, the ability of the uterus to release PGF is blocked. It is reasonable to suspect, therefore, that oxytocin may be involved in luteal regression in cycling mares and in maternal recognition of pregnancy in pregnant mares. However, circulating levels of oxytocin are much lower in mares than in sheep and cattle. If oxytocin is involved in luteal regression in mares, but the circulating levels were much lower in mares compared to ruminants, it seemed reasonable to look for a source of oxytocin close to the uterus. For example, if the uterus itself were producing oxytocin, then it could act directly on uterine PGF, without having to gain access to the systemic circulation.

From studies begun in 1992, we have been able to measure synthesis of both oxytocin protein (the biologically active molecule) and mRNA (the cellular molecules that act as a template for protein synthesis). To our knowledge this is the first evidence that the equine uterine endometrium produces oxytocin (Behrendt CY, Adams MH, Daniel KS, McDowell KJ. Oxytocin expression by equine endometrium. *Biol Reprod 1997*; 56 Suppl 1:134).

During the breeding season of 1996, endometrial biopsies were collected from mares in estrus, at days 5, 10 and 15 of a non-bred cycle, and at days 10, 15 and 20 of pregnancy. The amounts of mRNA appeared to be higher at estrus and late in the cycle, around the time of expected luteal regression, than earlier in the cycle, but there was a good deal of variation among animals. During the 1997 breeding season, more samples were collected and the studies are continuing.

When we quantitate changes in oxytocin mRNA and protein production by the endometrium, we will be able to compare these levels to those of uterine PGF, and investigate interactions between the two hormones. In this manner, we can gain a better understanding of luteal maintenance, luteal regression, and maternal recognition of pregnancy in mares.

The long-term goal of the research in our laboratory is to identify causes of pregnancy loss in horses and to develop methods of reducing the loss rates. The direct benefits to the equine industry are to increase efficiency and decrease costs of horse breeding programs.

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Salmonella Antimicrobial Susceptibility

In the October 1996 issue of the *Equine Disease Quarterly*, *Salmonella* isolates from equine cases over a six-year period at the Livestock Disease Diagnostic Center (LDDC) were discussed. From June 1994 to June 1996, 3340 equine necropsies on adults, neonates and fetuses were performed at the LDDC. *Salmonella* isolates from necropsy cases during the two years have been closely examined.

Dr. Marie Petrites-Murphy of the LDDC reviewed all cases to determine whether the *Salmonella* isolated was the primary cause of disease, as in cases of enterocolitis or septicemia, or the isolate was considered secondary or not a cause of disease (**Figure 1**). *Salmonella* can be found in the gut flora of normal horses, and was isolated in horses which died of head injury, neonatal isoerythrolysis, uterine hemorrhage and poisonings.

In assessing the 39 primary salmonellosis cases, *typhimurium* was the most frequently isolated serotype (16), followed by *newport* (8), *oranienburg* (3) and 8 other serotypes with 1 to 2 isolates each. The age distribution for the horses with *S. typhimurium* cases ranged from a 10-month fetus to a 16-year-old horse, with all animals coming from different farms. Ten animals were 6 months of age or less; 2 animals were 7 to 12 months of age and 4 horses were older than 12 months. The antibiotic susceptibilities of these 16 *S. typhimurium* isolates were examined: 15 were susceptible or moderately susceptible to 14 or 15 antimicrobial disks, and 1 from an adult horse with acute

Figure 1

***Salmonella* Isolates fro**
June 1994 to June 199

Salmon	
PRIMARY C	
39	
(*S. typhimuri*	
< 1 y	
≥ 1 y	
Salmon	
SECONDARY	
49	
(*S. typhimuri*	
< 1 y	
≥ 1 y	

colitis was susceptible only to polymyxin B, cephalothin, amikacin and ceftiofur.

Occasionally a single serotype predominated among clinical and necropsy cases for a short time, then disappeared. Of *Salmonella* isolates from central Kentucky clinical and necropsy cases during January 1990 to June 1996, *S. oranienburg* was cultured only in 1995 on 13 occasions. Seven isolates were from clinical cases (6 feces, 1 joint) and 6 were isolated at necropsy.

Post mortem findings indicated that 3 horses died or were euthanized due to primary *Salmonella* infections (1 severe osteomyelitis in a foal, 1 enterocolitis/septicemia in a foal, and 1 enteritis from a 20-year-old horse). Seven isolates came from horses on one farm during a 3 month period (4 feces, 2 intestinal contents and 1 joint). One foal tested *S. oranienburg* positive on fecal culture, was euthanized due to suppurative arthritis, and *oranienburg* was cultured from a draining tract in the leg. Both isolates had identical antibiotic susceptibility patterns with resistance to neomycin, kanamycin, sulfa drugs, tetracycline and tribrissen. Of the other 5 isolates from this farm's horses, 4 were susceptible to virtually all 15 antibiotics tested, and 1 had antibiotic resistance to 6 antibiotics which differed from the resistance pattern of the isolate from the first foal. Even in animals from the same farm over a short period of time, one *Salmonella* serotype can have various antibiotic susceptibility patterns.

Antibiotic resistance can be a significant problem in veterinary hospital salmonellosis outbreaks in the United States. One equine hospital occurrence involved horses infected with *S. typhimurium* which was susceptible only to ceftiofur and amikacin. Although this outbreak resulted in several equine deaths, no human cases of disease were reported. In another outbreak, *Salmonella infantis* was identified in a veterinary hospital causing high morbidity, but low mortality in large animals, primarily horses. Infections occurred from January through September 1996. At the onset of the problem the organism was resistant to 5 antimicrobials in a susceptibility panel. After May 1996 the majority of the isolates were resistant to 8 antimicrobials and only susceptible to amikacin and enrofloxacin.

All salmonellae which affect horses can potentially cause disease in humans and other exposed animals. Strict isolation of clinically ill horses, proper disposal of feces and bedding, thorough disinfection of facilities and equipment, use of protective clothing, and frequent handwashing can help prevent the spread of salmonellosis to other horses, animals and people.

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K E N T U C K Y

Tyzzer's Disease

Tyzzer's disease affects many species of animals. It was originally described as an illness of mice, but has also been reported to cause disease in dogs, cats, rats, gerbils, rabbits, guinea pigs, monkeys, muskrats, hamsters, and foals. Tyzzer's disease is caused by a bacterium named *Clostridium piliforme (Bacillus piliformis)*. This organism is an obligate intracellular pathogen and can be grown only in tissue cultures.

The disease in horses occurs as an acute, typically fatal disease of foals around one month of age. The foal is usually found dead but clinical signs of depression, fever, and jaundice may be seen. When examined pathologically, the liver is often swollen with areas of hemorrhage and necrosis. Microscopically, the liver lesions consist of areas of necrosis with little inflammatory response. The bacteria can be seen inside hepatocytes at the periphery of the necrotic zones.

Over the last 5 years, 27 cases of Tyzzer's disease have been diagnosed in foals at the Livestock Disease Diagnostic Center **(Figure 2)**. This number represents 2% of the total foals in this age range that were necropsied. There were 13 colts and 13 fillies and one case where the sex of the foal was not noted. Of the 27 total cases, 25 were Thoroughbreds with the other 2 cases being a Standardbred and a Quarterhorse. The high percentage of Thoroughbreds is consistent with the overall breed distribution

seen at this facility. The ages of the foals ranged from 7 to 92 days old with an average age of 25 days. Only 3 foals were greater than 30 days of age. All 27 cases died in the months of February through June. No case was received after June 10th.

Cases were from different farms with one exception. This farm was the only one with cases during several years, 1 in 1993, 2 in 1996 and 3 in 1997.

Unfortunately, since there is no test to diagnose this disease in live foals, a definitive diagnosis of Tyzzer's disease can be made only on post mortem examination. The manner by which this disease is spread is not known. It is believed that the most likely means of spread is by fecal-oral transmission. As our findings indicate, this disease is primarily sporadic which suggests that it is not highly contagious among horses. However, as shown here and reported elsewhere, individual farms can have clusters of cases. The reasons for this are not clear, but based on this observation, foals born on farms with a history of Tyzzer's disease should be carefully monitored for the first 60 days of life.

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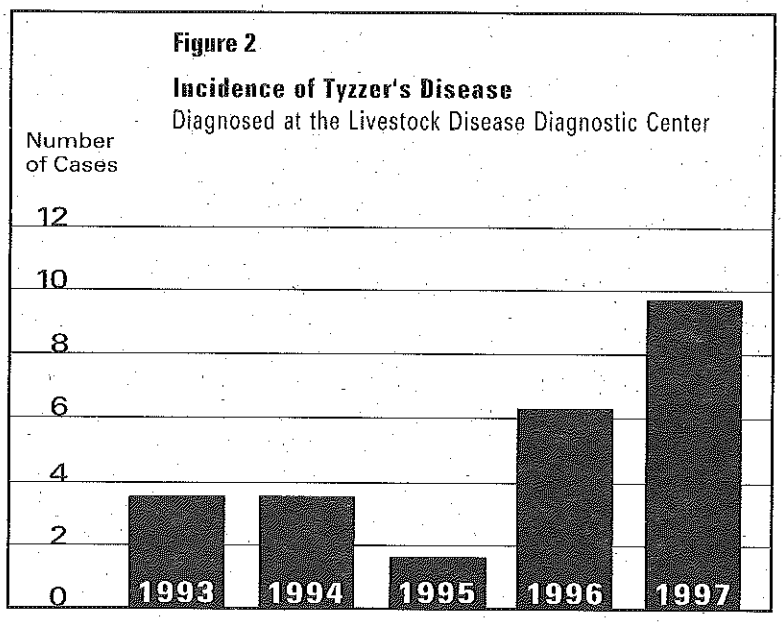
Figure 2

Incidence of Tyzzer's Disease
Diagnosed at the Livestock Disease Diagnostic Center



Fescue Toxicosis

If you suspect that your horse or horses are showing signs of fescue toxicosis such as agalactia, prolonged gestation, dystocia or foaling difficulty or thickened placenta ("red bag" foal), follow these steps to help determine if in fact fescue is the cause.

Fescue may enter the horse diet either from pasture, grain mix, bedding or supplemental hay. It is important to characterize all of these to determine the source of the problem. The toxic compounds in tall fescue are present in very small quantities (parts per million and parts per billion levels) and in some cases are destroyed by the way that samples are handled.

To fully determine the likely cause of the toxicosis, all aspects of the forage components have to be analyzed. In many cases, commercial assays for the toxic compounds are expensive and slow. Therefore, in most cases it will not be possible to get a complete characterization quickly.

Pasture—Locate and identify fescue in the pasture fields that the pregnant mare was grazing. Make notes on the relative percentage of the field that is in fescue and its height relative to the other grasses.

To determine if the fescue is infected with the endophyte, 2 x 2 x 2 inch "divot" samples of the fescue are required that have approximately 1 inch of soil and 1 inch of grass. Take approximately 20 for a 10-acre field of fescue. Keep these hydrated and submit them to your local county extension office or to Regulatory Services on the University of Kentucky campus (immediately south of the Gluck Research Center at the corner of Alumni Drive and University Drive). There is a fee for this service based on the number of samples taken (usually around $20).

This assay is most accurate when fescue is actively growing, usually during the months of April, May and June. At other times, other types of tests must be used.

Grain mix—If supplemental feed was being fed, take samples of this material for analysis. Some feed can be contaminated with ergot fungal bodies which cause the same symptoms as fescue toxicosis.

Bedding— It is common to bed horses on pasture clippings which may have been taken from fields that contained significant amounts of fescue. If horses were bedded on this type of material, determine if

KENTUCKY CONTINUED

fescue was present. Fescue leaves will most likely be rolled up when present in bedding or hay, making identification more difficult. The alkaloids in fescue are still toxic in hay or clippings made from infected plants, especially if seed heads are present.

Supplemental hay—Check a flake of the hay being fed for the presence of fescue. However, be aware that many grasses may look similar when cured into hay and fescue may not be definitely identified.

Other sources—In some cases, used bedding from other barns is spread onto pastures used by pregnant mares. If this is the case, determine if fescue is present.

The following are good sources of information about fescue and the endophyte:

Fescue for Horses: Problem or Opportunity? D. Ball, M. Putnam, G. Lacefield, and C. Hoveland. Special Publication, Oregon Tall Fescue Commission, Salem, Oregon. Available from Jimmy Henning (606) 257-3144, Department of Agronomy, N-222D Ag Science North, University of Kentucky, Lexington, Kentucky 40546-0091.

Alternatives for fungus infected tall fescue. G. Lacefield and J. Henning. UK Cooperative Extension Service, AGR 119.

Sampling for the tall fescue endophyte in pasture or hay stands. P. Vincelli, M. Siegel, and E. Fabrizius. UK Cooperative Extension Service, PPA 30.

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