



2011's Top Equine Studies

ERICA LARSON, CHRISTY WEST

Each year, researchers publish hundreds of horse health studies. And each year at the convention, three practitioners have the unique (and behemoth) task of deciding which to feature during the Kester News Hour.

This time Patrick M. McCue, DVM, PhD, Dipl. ACT, associate professor of equine science at Colorado State University in Fort Collins; Scott E. Palmer, VMD, Dipl. ABVP (Equine Practice), hospital director and staff surgeon at New Jersey Equine Clinic in Clarksburg, N.J.; and Steve Reed, DVM, Dipl. ACVIM, associate at Rood & Riddle Equine Hospital in Lexington, Ky., presented the top studies in equine reproduction, surgery, and medicine, respectively.

The hosts of the news report-themed presentation described the studies they found most important, interesting, or applicable during the session that is a perennial favorite among veterinarians. Here is a sampling of the research they covered. Journal references for these studies and summaries of additional studies can be found online (see links on page 15).

Metabolic & Endocrine Disorders

Reed began by describing a handful of studies on diagnosing the single most commonly detected endocrine disorder in aging horses—**pituitary pars intermedia dysfunction** (PPID, or equine Cushing's disease), which is characterized by enlargement of the pars intermedia region of the pituitary gland. Diagnosing PPID, he noted, has been a challenge in the past, and usually affected horses are identified through a combination of age and clinical signs.

In one study Reed referenced, researchers evaluated which breeds are most prone to PPID diagnosis and what—if any—effect geographic location had on PPID diagnosis. About 36% of all horses are diagnosed with PPID, Reed noted, but the disease is most commonly found in pony breeds and Morgan horses.

Further, the researchers found that levels of two hormones released by the pars



From left to right: Drs. Patrick M. McCue, Scott E. Palmer, and Steve Reed presented the top studies in equine reproduction, surgery, and medicine, respectively.

intermedia—adrenocorticotrophic hormone (ACTH) and α -melanocyte-stimulating hormone (α -MSH)—increase in all horses in the fall. And while fall begins earlier in the North than in the South, horses in the South display a greater pars intermedia response to the change in season.

The study is a step toward fulfilling certain diagnostic needs, he noted: “Seasonal and photoperiod (daylight’s effect on plants and animals) reference ranges are needed when interpreting pars intermedia hormones in horses and may be helpful for early recognition of PPID in horses.”

Additionally, Reed discussed a study in which a research team evaluated **ACTH levels** in more than 900 horses with PPID and another 900 without PPID. They found that during the fall (August to October) horses with PPID had an average ACTH concentration of 120 pg/mL compared to November to July when the levels were only 60 pg/mL. While levels fluctuated throughout the year in the non-PPID horses as well, the average ACTH thresholds were significantly

lower year round in normal horses.

Reed concluded that because of the variation in hormone concentrations year-round, the fall could be the best time to test as a result of the observed spike in ACTH levels in PPID horses.

Respiratory & Cardiovascular Topics

Reed changed gears, describing a study in which scientists evaluated **the effect of furosemide** (commonly known as Salix) on treating exercise-induced pulmonary hemorrhage (“bleeders”). They found that administering 250 mg of Salix four hours prior to exercise (they tested the horses at 80% of maximum energy output) did not alter the horses’ exercise performance, and it did not impact the way capillaries in the lungs regulated red blood cell volume, despite the fact it decreased cardiac output. Reed noted researchers are still working to understand exactly where in the lungs the bleeding originates and that it’s possible a higher dose of Salix could have yielded different results.



Veterinarians often treat another respiratory disorder, **recurrent airway obstruction** (RAO, heaves), with systemic corticosteroids. This treatment method is often effective, but prolonged use can have adverse effects (including a suppressed immune system and an increased risk of founder) in some horses. Reed discussed a study in which researchers examined the effects of using an inhaled corticosteroid to treat RAO as opposed to traditional administration methods. The team assessed effects on the two arms of the immune systems, cell-mediated (protecting against intracellular organisms) and humoral immunity (via antibodies in the serum that protect the horse from infection), and noted no adverse reactions. Further, the team found that prolonged administration yielded no undesirable results.

Diagnostic Techniques

Reed then delved into a few studies regarding new or updated diagnostics. He first discussed a study in which investiga-

tors determined that **dehydration in horses** led to abnormalities evident on echocardiographic examination (an ultrasound of the heart). The most significant changes were observed in the left ventricle (the lower chamber of the heart that pumps oxygenated blood throughout the body) and left atrium (the upper chamber that transports the oxygenated blood from the pulmonary veins into the left ventricle) of dehydrated horses, both of which appeared decreased in size. The thickness of some wall structures within the heart also increased.

"The researchers warned (dehydration) could result in alteration of variables often applied to predict athletic potential in a horse," Reed said.

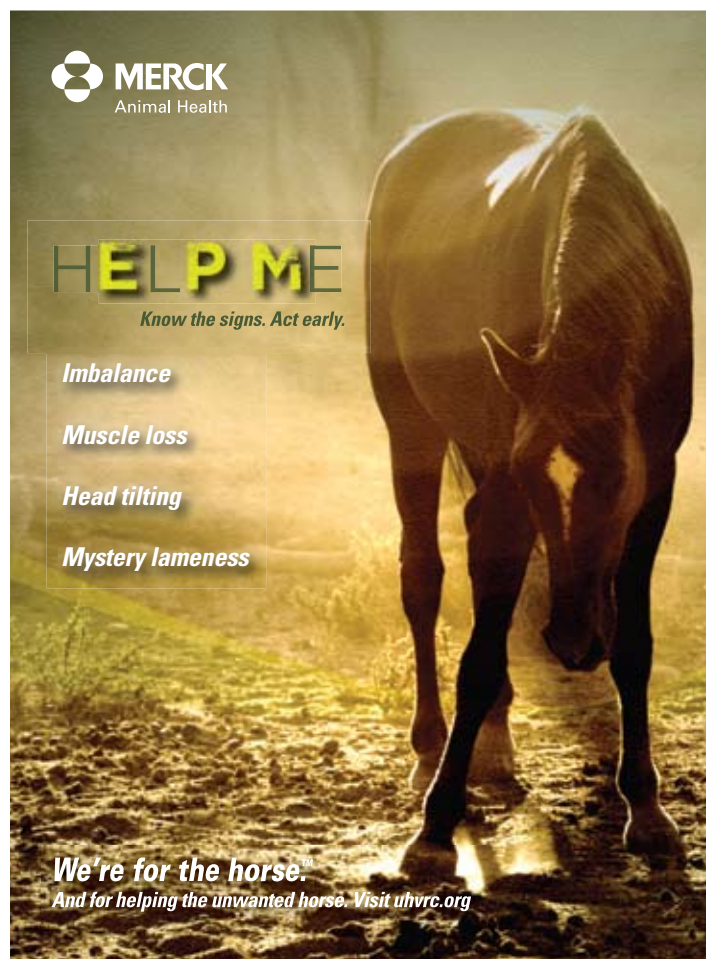
Clinicians are diagnosing **cervical (neck vertebrae) problems in horses** more frequently. While clinical assessment and cervical vertebral radiographs are the usual diagnostic tools veterinarians use, Reed discussed a study in which researchers examined the use of electromyography (which measures electrical activity of


muscles) for diagnosis. Often used in human medicine for diagnosing movement disorders, the team tested the technique in horses and found it useful for evaluating suspected cervical lesions.

"(The researchers) seek to establish normal values to use for the diagnosis of horses with neurological problems involving the cervical vertebrae," he said, which will help vets recognize what is abnormal.

Drugs and Side Effects

Reed relayed the results of a recent study in which a research team reviewed **non-steroidal anti-inflammatory drugs' (NSAIDs) effects on equine intestines**; he noted veterinarians have observed side effects including gastric ulceration and right dorsal colitis, especially when using these drugs to treat endotoxemia. Through their research, the team confirmed that extended NSAID use damages the intestinal mucosal barrier, which leads to a loss of normal barrier function (against some common antigens and pathogens).



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Ocular Health

Reed closed his segment by discussing a study in which researchers evaluated the presence of **ophthalmic lesions in neonatal foals** admitted to a referral hospital for unrelated reasons. Fifty-six percent of foals had at least one potentially vision-limiting ophthalmic disease (possibly acquired as a result of another health problem).

He said, "Don't forget to look at the entire foal. If you treat them early, you might find things that could save their vision."

EVA Vaccines

McCue described equine viral arteritis (EVA) as a highly contagious disease that can cause weakness or sickness in foals and abortion in broodmares. The virus can be transmitted via respiratory secretions or breeding (natural cover or artificial insemination). The AAEP's vaccination guidelines state, "The manufacturer does not recommend use of (the EVA) vaccine in pregnant mares, especially in the last two months of pregnancy." However, a team of researchers revisited **the safety of vaccinating pregnant mares against EVA**. McCue summarized, "It appears ... safe to vaccinate healthy pregnant mares against equine arteritis virus up to three months before foaling and during the immediate postpartum period. Vaccination during the last two months of pregnancy was associated with a risk of abortion."

Veterinarians also recommend **EVA vaccination for stallions** (an initial vaccine, then annual boosters), but researchers on a recent study found a temporary low level of viral activity in semen shortly after initial vaccination.

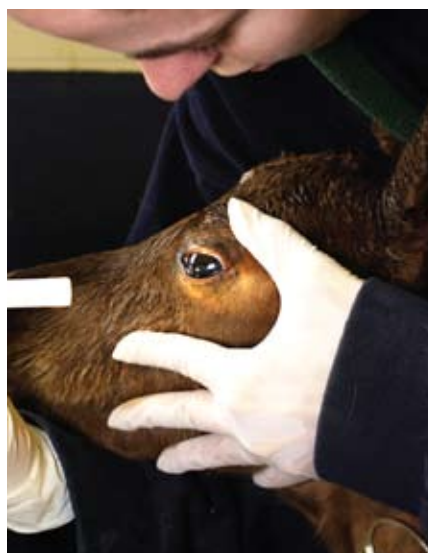
"Withhold use of semen for 14 days after first-time vaccination to minimize risk of transmission of vaccine virus and eliminate potential for vaccine virus in frozen semen," advised McCue. After the initial low level viral activity, there was no evidence of persistent vaccine virus in first-time vaccinated stallions' reproductive tracts, and there was no transmission of vaccine virus to in-contact horses.

Sperm Morphology and Quality

"Assessment of sperm morphology (physical characteristics) is a key component of semen analysis," noted McCue as he described a study in which researchers compared **semen evaluation staining procedures** for use in horses.

The scientists found wet mount preparations to be more sensitive for detecting abnormal sperm than stained smears, while smear preparations increased the proportion of detached heads. The Papanicolaou stain (a multichromatic staining that allows scientists to differentiate cells in smear preparations; it's well-known for its use cervical cancer screening in women) was unsuitable for stallions.

"A significant difference was noted among clinicians, suggesting that consistent training is important," he explained.



Reed said veterinarians should be sure to examine sick foals' eyes to detect possible associated eye illness.

In another **semen quality and fertility study** scientists looked at seasonal pregnancy rate, percentage of mares pregnant per cycle, and percentage pregnant on the first cycle. They found that a number of sperm abnormalities (such as coiled tails or abnormal/detached heads) depressed fertility, and more fertile stallions had better sperm motility and a higher percentage of morphologically normal sperm.

"The percentage of mares pregnant on their first cycle was the only fertility measurable to discriminate among high, average, and low fertility groups (among stallions)," McCue noted.

Embryo Transfer

McCue described one report of a successful embryo transfer program as "a valuable contribution to the world of embryo transfer with mind-boggling numbers—a great data set." Investigators presented the techniques and lessons learned from near-

ly 15,000 embryo flush procedures (up to 2,300 per year), nearly 8,000 established pregnancies, and 1,900 embryos transferred over a 13-year period.

"The take-home message is that a **breeding soundness evaluation** should be performed on each donor and recipient mare," summarized McCue. "Also, recipient mare selection and management is critical to success. Recipient mares can ovulate anywhere from the day before the donor mare ovulates until four days after the donor mare ovulates.

"Freezing small embryos is easy, but large ones are problematic and historical failures," said McCue as he introduced a study of **a new technique for optimizing freezing of large embryos by the process of vitrification**. This technique involves removing fluid from the initially hollow early-stage embryo (termed a blastocyst) to collapse it prior to vitrification. Large embryos that were intentionally collapsed prior to freezing yielded good pregnancy rates after transfer (there was a 71% pregnancy rate in this study).

"This technique appears to be a significant step forward for equine embryo transfer and (embryo) cryopreservation," McCue commented.

Veterinarians might biopsy embryos for genetic evaluation for several reasons, from determining sex to identifying inherited diseases. He discussed a study in which scientists defined proper embryo biopsy techniques and ways to evaluate results.

"**Biopsy of large blastocyst stage embryos** was possible and did not compromise embryo viability," he reported. "Genetic analysis was accurate for sex determination and is a 'work in progress' for diagnosing HERDA (hereditary regional dermal asthenia, also called hyperelastosis cutis, a skin condition) and HYPP (hyperkalemic periodic paralysis, a metabolic/muscle condition). Pre-implantation genetic diagnosis may soon become a viable clinical procedure in the horse."

Respiratory Problems: Surgical Approach

"Coughing is a common complaint in mature nonracehorses with epiglottic abnormalities," began Palmer as he described a retrospective study of mature horses (mean age of 16, with a range from 9 to 30) with **abnormalities of the epiglottis** (a flap of cartilage that moves to cover a horse's



windpipe when he swallows to prevent inhaling fluid or food). In the 23 horses studied, the primary complaint for 70% was chronic cough and the most common epiglottic abnormality was epiglottic entrapment, which results in hampered epiglottal movement (followed by subepiglottic granuloma and subepiglottic cyst). All horses were treated surgically and 74% required no further treatment, while a few required additional treatment for inflammation, RAO, and dorsal displacement of the soft palate.

"Upper airway endoscopy is recommended in the evaluation of older horses with a cough," summarized Palmer. "Surgical treatment can be beneficial in most horses, with some requiring further postoperative medical treatment."

In the years that have passed since the release of a practical backpack-based endoscopic system for examining the horse's airway during normal exercise, researchers have been reevaluating accepted knowledge about equine respiratory problems and working to develop optimal workouts for revealing abnormalities.

Another group of researchers recently published their investigation of **dynamic endoscopy exams** and the workouts used during those exams. Notably, airway noise was the easiest condition to reproduce on examination, and poor performance without noise was most difficult.

"A principal advantage of dynamic videoendoscopy is the ability to evaluate the horse in its natural surroundings and under race conditions," Palmer said. "However, it may be difficult to standardize exercise tests in the field under widely varying conditions. It is best to replicate race conditions as closely as possible with peers (working alongside); no single protocol is likely to be appropriate for all exams."

Laryngoplasty, also called tieback surgery, is a relatively common treatment for horses that make significant airway noise during exercise (roaring). The surgery—which involves placing one or two sutures in the left arytenoid cartilage to hold it out of the airway—is usually effective, but as with most treatments its success rate isn't 100%. A research team examined records of 45 horses that had had left laryngoplasty surgery over a 15-year period to see if collapse of the cartilage after surgery could be predicted from post-procedure resting endoscopy, and to explain why some

horses perform poorly after surgery.

Palmer reported that horses with no post-surgical abduction (opening) tended to have left arytenoid collapse, but horses with moderate or good degree of abduction showed no predisposition to collapse.

"Complex obstructions causing respiratory noise were observed in most examinations, but they were not specific for arytenoid collapse," Palmer added. "These data underscore the value of performing a dynamic examination prior to surgery in order to identify conditions that may compromise the outcome of laryngoplasty."

Sinus infections in horses can be just as tough to get rid of as those in humans. Palmer reviewed one study in which veterinarians documented long-term health of horses following conservative treatment (such as standing procedures to drain or remove pus from and rinse the sinuses) for various types of sinus disease.

Long-term results were mostly positive, with 91% of horses cured and usually after only one treatment. The exception was sinus neoplasia (tumors), in which just 22% were cured.

“Pre-implantation diagnosis (of inherited diseases) may soon become a viable clinical procedure in the horse.”

DR. PATRICK M. MCCUE

"More conservative treatments, including removal of intrasinus inspissated (thickened) pus by sinuscopy, pre-existing sinonasal fistula, or sinusotomy, (three procedures) which avoid the risk and expense of general anesthesia are effective in managing chronic primary sinus disease in many cases," reported Palmer.

Colic

"**Recurrent colic** is often discussed but not well-documented," noted Palmer, as he described a study on the condition and risk factors for it. In this study nearly 37% of horses with medically treated colic had another colic episode within the next year.

"Recurrence of colic was higher than previously reported," he commented. "Horses with a known dental problem or

cribbing/windsucking were at increased risk of recurrence. This confirms long-held beliefs and gives us a baseline number to share with clients when treating medical colics in the field."

Diagnosing strangulating bowel lesions associated with colic isn't always easy; the horse's torso is quite large and capable of "hiding" some deep lesions such as bowel twists severe enough to cut off blood flow. However, a recent study provided insight on a simple test that can help identify strangulating bowel lesions requiring immediate surgical intervention.

In the study of 94 horses with colic, researchers found rising concentrations of lactate in peritoneal (abdominal cavity) fluid sampled via a "belly tap" over a 30-minute period to be a "good indicator of strangulating lesions, easily performed, sensitive (79%), specific (88%), and a good indicator of the need for surgery," said Palmer. "Unrelenting pain will always be a primary factor, but this test can help in more ambiguous cases."

Stem Cells

An increasing number of veterinarians are using stem cell therapy, and rising with this trend is the need for a **safe, efficient bone marrow-derived stem cell harvesting technique**. In a recent study authors described such a technique that involves placing a Jamshidi needle (a cylindrical needle with a tapered cutting tip) into the fifth sternebra (a segment of the sternum) with ultrasound guidance. Palmer noted that this procedure "enables aspiration of bone marrow reliably with minimal damage to the sternum and risk to the horse."

"Long-term follow-up (after stem cell usage) is hard to find," said Palmer. He discussed a study of stem cell usage in which the researchers observed 141 racing Thoroughbreds (flat and steeplechase) for at least two years after returning to full work following stem cell treatment of superficial digital flexor tendon injuries.

"Tendon lesions filled in quickly, histological (tissue architecture) appearance showed well-aligned fibrils, and there was an absence of scar tissue," he described. "Ninety-eight percent returned to racing with a 26% re-injury rate in hunt horses and 50% in flat horses. There was no correlation between outcome and age, discipline, number of stem cells injected, or injury-to-implantation interval."

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"This study shows that marrow-derived mesenchymal stem cell implantation is safe and appears to reduce re-injury rate after superficial digital flexor injury, especially in (steplechase horses)," he concluded. "It is the first report that provides evidence for long-term efficacy of mesenchymal stem cell treatment for tendinopathy (tendon disease) in racehorses. However, the long-term success in the Thoroughbred flat horse remains a challenge."

To successfully treat lesions stem cells need to reach and stay in the lesion, and the administration route (intravenous, intralesional, or regional perfusion) has a lot to do with that. Researchers recently compared the **three methods of administering stem cells** and found that intral-lesional injection of stem cells (directly



COURTESY DR. GAIL KEMLER

Regional limb perfusion was one of the methods scientists examined for administering stem cells. It came in second place to intralesional injection.

into the lesion) was the most effective for keeping stem cells within the lesion after 24 hours (the lesion retained 10% of cells), while regional perfusion came in a close second with cells remaining in the lesions of 11 of 12 horses. In contrast, stem cells injected intravenously did not "home" on the lesions at all, instead scattering mostly around the lungs.

"Regional perfusion is a viable alternative if no core lesion (single obvious injury) is present," commented Palmer.

When injecting stem cells it might seem logical to also inject a little antibiotic to prevent infection from developing due to any pathogens introduced during the process. However, the clear message from a

recent study is that this can be a very bad idea: **Don't mix stem cells with gentocin or amikacin antibiotic**, as they kill the stem cells. In this study researchers found that those two antibiotics killed more than 95% of the injected stem cells after 45 minutes and two hours, respectively.

However, incubating stem cells with hyaluronic acid and penicillin/streptomycin resulted in acceptable viability of 80%.

Treated Infected Joints

"Septic arthritis is commonly treated with intravenous (IV) and regional perfusion in conjunction with joint lavage (flushing)," began Palmer as he discussed a study of tourniquet types. For regional limb perfusion, a veterinarian places a tourniquet on the leg for a short time to stop blood from flowing back up the limb before injecting antibiotic below the tourniquet. This allows the antibiotic to concentrate at high levels in the bloodstream below the tourniquet, perfusing the tissues.

“Upper airway endoscopy is recommended in the evaluation of older horses with a cough.”

DR. SCOTT PALMER

Theoretically, he noted, **performing a joint lavage simultaneously with regional limb perfusion** could cause antibiotic loss in the joint and compromise treatment.

"In standing horses, IV regional limb perfusion performed simultaneously with joint lavage resulted in negligible loss of amikacin in the egress lavage fluids (the less antibiotic lost, the better)."

Palmer reported, "This study validated the positive effect of the simultaneous use of these two treatments for joint infection. Additionally, the Esmarch tourniquet was more effective in preventing loss of amikacin from the distal portion of the limb, easier to use, and less expensive than the pneumatic tourniquet." 🐾

MORE ONLINE See TheHorse.com/AAEP2011 for study references and for more research.

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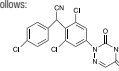
For the treatment of equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona* in horses.

CAUTION Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian.

NADA #141-268 Approved by FDA

DESCRIPTION

Diclazuril, (±)-2,6-dichloro-α-(4-chlorophenyl)-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl) benzeneacetamide, has a molecular formula of C₁₇H₁₁Cl₃N₃O₂, a molecular weight of 407.64, and a molecular structure as follows:



Diclazuril is an antiprotozoal (antiparasitic) compound with activity against several genera of the phylum Apicomplexa. PROTAZIL® (diclazuril) is supplied as oral pellets containing 1.56% diclazuril to be mixed as a top-dress in feed. Inert ingredients include dehydrated alfalfa meal, wheat middlings, cane molasses and propionic acid (preservative).

INDICATIONS

PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets are indicated for the treatment of equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona* in horses.

DOSEAGE AND ADMINISTRATION

Dosage: PROTAZIL® (1.56% diclazuril) is administered as a top-dress in the horse's daily grain ration at a rate of 1 mg diclazuril per kg (0.45 mg diclazuril/lb) of body weight for 28 days. The quantity of PROTAZIL® necessary to deliver this dose is 64 mg pellets per kg (29 mg pellets/lb) of body weight.

Administration: To achieve this dose, weigh the horse (or use a weigh tape). Scoop up PROTAZIL® to the level (cup mark) corresponding to the dose for the horse's body weight using the following chart:

Weight Range of Horse (lb)	mLs of Pellets	Weight Range of Horse (lb)	mLs of Pellets
275 - 524	20	1275 - 1524	60
525 - 774	30	1525 - 1774	70
775 - 1024	40	1775 - 2024	80
1025 - 1274	50	-	-

One 2-lb bucket of PROTAZIL® will treat one 1100-lb horse for 28 days. One 10-lb bucket of PROTAZIL® will treat five 1100-lb horses for 28 days.

CONTRAINDICATIONS Use of PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets is contraindicated in horses with known hypersensitivity to diclazuril.

WARNINGS

For use in horses only. Do not use in horses intended for human consumption. Not for human use. Keep out of reach of children.

PRECAUTIONS

The safe use of PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets in horses used for breeding purposes, during pregnancy, or in lactating mares has not been evaluated. The safety of PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets with concomitant therapies in horses has not been evaluated.

ADVERSE REACTIONS

There were no adverse effects noted in the field study which could be ascribed to diclazuril. To report suspected adverse reactions, to obtain a MSDS, or for technical assistance call 1-800-224-5318.

CLINICAL PHARMACOLOGY

The effectiveness of diclazuril in inhibiting merozoite production of *Sarcocystis neurona* and *S. falcatula* in bovine turbinate cell cultures was studied by Lindsay and Dubey (2000). Diclazuril inhibited merozoite production by more than 80% in cultures of *S. neurona* or *S. falcatula* treated with 0.1 ng/mL diclazuril and greater than 95% inhibition of merozoite production (IC₅₀) was observed when infected cultures were treated with 1.0 ng/mL diclazuril. The clinical relevance of the in vitro cell culture data has not been determined.

PHARMACOKINETICS IN THE HORSE

The oral bioavailability of diclazuril from the PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets at a 5 mg/kg dose rate is approximately 5%. Related diclazuril concentrations in the cerebrospinal fluid (CSF) range between 1% and 5% of the concentrations observed in the plasma. Nevertheless, based upon equine pilot study data, CSF concentrations are expected to substantially exceed the in vitro IC₅₀ estimates for merozoite production (Dirikolu et al., 1999). Due to its long terminal elimination half-life in horses (approximately 43-65 hours), diclazuril accumulation occurs with once-daily dosing. Corresponding steady state blood levels are achieved by approximately Day 10 of administration.

EFFECTIVENESS

Two hundred and fourteen mares, stallions, and geldings of various breeds, ranging in age from 6 months to 30 years, were enrolled in a multi-center field study. All horses were confirmed EPM-positive based on the results of clinical examinations and laboratory testing, including CSF Western Blot analyses. Horses were administered PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets at doses of 1, 5, or 10 mg diclazuril/kg body weight as a top-dress on their daily grain ration for 28 days. The horses were then evaluated for clinical changes via a modified Mayhew neurological scale on Day 48 as follows:

1. Neurological deficits not detected.
2. Neurological deficits may be detectable at normal gaits; signs exacerbated with manipulative procedures (e.g., backing, turning in tight circles, walking with head elevation, trunk sway, etc.).
3. Neurological deficit obvious at normal gaits or posture; signs exacerbated with manipulative procedures.
4. Neurological deficit very prominent at normal gaits; horses give the impression they may fall (but do not) and buckle or fall with manipulative procedures.
5. Neurological deficit is profound at normal gait; horse frequently stumbles or trips and may fall at normal gaits or when manipulative procedures were utilized.
6. Horse is recumbent, unable to rise.

Each horse's response to treatment was compared to its pre-treatment values. Successful response to treatment was defined as clinical improvement of at least one grade by Day 48: a conversion of CSF to Western Blot-negative status for *S. neurona* or achievement of Western Blot-negative CSF status without improvement of 1 ataxic grade.

Forty-two horses were initially evaluated for effectiveness and 214 horses were evaluated for safety. Clinical condition was evaluated by the clinical investigator's subjective assessment and then corroborated by evaluation of the neurological examination videotapes by a masked panel of three equine veterinarians. Although 42 horses were evaluated for clinical effectiveness, corroboration of clinical effectiveness via videotape evaluation was not possible for one horse due to missing neurologic examination videotapes. Therefore, this horse was not included in the success rate calculation.

Based on the numbers of horses seroconverted to negative Western Blot status, and the numbers of horses classified as successes by the clinical investigators, 28 of 42 horses (67%) at 1 mg/kg were considered successes. With regard to independent expert masked videotape assessments, 10 of 24 horses (42%) at 1 mg/kg were considered successes. There was no clinical difference in effectiveness among the 1, 5, and 10 mg/kg treatment group results. Adverse events were reported for two of the 214 horses evaluated for safety. In the first case, a horse was enrolled showing severe neurologic signs. Within 24 hours of dosing, the horse was recumbent, biting, and exhibiting signs of dementia. The horse died, and no cause of death was determined. In the second case, the horse began walking stiffly approximately 13 days after the start of dosing. The referring veterinarian reported that the horse had been fed grass clippings and possibly had laminitis.

ANIMAL SAFETY

PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets were administered to 30 horses (15 males and 15 females, ranging from 5 to 9 months of age) in a target animal safety study. Five groups of 6 horses each (3 males and 3 females) received 0, 5 (5X), 15 (15X), 25 (25X) or 50 (50X) mg diclazuril/kg (2.27 mg/lb) body weight/day for 42 consecutive days as a top-dress on the ration of the horse. The variables measured during the study included: clinical and physical observations, body weights, food and water consumption, hematology, serum chemistry, urinalysis, fecal analysis, necropsy, organ weights, gross and histopathologic examinations. The safety of diclazuril top-dress administered to horses at 1 mg/kg once daily cannot be determined based solely on this study because of the lack of an adequate control group (control horses tested positive for the test drug in plasma and CSF). However, possible findings associated with the drug were limited to elevations in BUN, creatinine, and SDH and less than anticipated weight gain.

Definitive test article-related effects were decreased grain/top-dress consumption in horses in the 50 mg/kg group.

In a second target animal safety study, PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets were administered to 24 horses (12 males and 12 females, ranging from 2 to 8 years of age). Three groups of 4 horses/sex/group received 0, 1 or 5 mg diclazuril/kg body weight/day for 42 days as a top-dress on the grain ration of the horse. The variables measured during the study included: physical examinations, body weights, food and water consumption, hematology, and serum chemistry. There were no test article-related findings seen during the study.

STORAGE INFORMATION

Store between 15°C to 30°C (59°F to 86°F).

HOW SUPPLIED PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets are supplied in 2-lb (0.9 kg) and 10-lb (4.5 kg) buckets.

REFERENCES

1. Lindsay, D. S., and Dubey, J. P. 2000. Determination of the activity of diclazuril against *Sarcocystis neurona* and *Sarcocystis falcatula* in cell cultures. *J. Parasitol.* 86(1):164-166.
2. Dirikolu, L., Lehner, F., Natrass, C., Bentz, B. G., Woods, W. E., Carter, W. E., Karplesik, W. G., Jacobs, J., Boyles, J., Harkins, J. D., Granstrom, D. E., and Tobin, T. 1999. Diclazuril in the horse: Its identification and detection and preliminary pharmacokinetics. *J. Vet. Pharmacol. Ther.* 22:373-379.

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