General Medicine

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EHV-1 Vaccine Efficacy Tested

Equine herpesvirus-1 (EHV-1) is highly prevalent in the horse population due to its ability to persist latently in infected animals for their lifetimes, where it can be reactivated and shed in times of stress. Although veterinarians cannot eliminate this virus entirely, they can make efforts toward containment. Immunization is one pivotal strategy in controlling virus spread in an equine population, and an immunologist from Colorado State University described efficacy of two available EHV-1 vaccines.

In his presentation Paul Lunn, DVM, BVSc, MS, Dipl. ACVIM, of the College of Veterinary Medicine and Biomedical Sciences, described the classic characterization of an EHV-1 infection with its two-phase fever spike; an initial fever is associated with nasal shedding and the second fever coincides with viremia (virus circulating in the bloodstream). Then by seven to 12 days, sequelae such as abortion or neurologic signs appear.

Veterinarians have demonstrated in past studies that the best available vaccines were somewhat effective in limiting nasal shedding, but they were only sometimes effective in preventing development of viremia. Lunn commented that vaccines protected best when horses had received boosters recently. Best results also were achieved when vaccines labeled against herpes viral abortion were used—vaccines labeled solely for respiratory EHV prevention have only 30% as much antigen concentration as the anti-abortigenic vaccines.

He described a challenge study he and colleagues performed on three groups of 10 young ponies (2 ½ years old). Each of two groups was immunized with either of two commercially available killed-virus vaccines (Prodigy by Intervet—now Merck Animal Health—and Calvenza by Boehringer Ingelheim). Horses in the third group served as nonvaccinated controls. The researchers gave the initial two priming doses of herpes vaccine one month apart; they delayed the third vaccine in the primary series another three months, and then they subjected the ponies to viral challenge one month after the third immunization in the series.

The challenge experiment did not produce extensive disease, which Lunn suggested could in part be due to the ponies' young age. While clinical scores, rectal

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Currently available equine herpesvirus-1 vaccines can help reduce nasal shedding of the virus and, hence, exposure within a herd or population of horses.

temperatures, viral shedding, and viremia for the three groups were similar, investigators noted statistical differences in virus neutralization titers between the vaccination groups: Calvenza-immunized ponies had higher titers than the Prodigy-immunized group, whose titers were higher than the nonvaccinated control ponies.

Lunn concluded, "There was no significant reduction of clinical signs or nasal shedding by either vaccine, only a trend." He emphasized that the best killed and live vaccines are somewhat effective only if regular vaccine regimes are implemented along with timely boosters.

While currently available vaccines might not protect directly against neurologic sequelae to herpesvirus infection, such as equine herpes myeloencephalopathy, there is value in using the highly antigenic (antibody-stimulating) herpes vaccines. Their value lies in reducing nasal shedding of virus and, hence, exposure within a population of horses that is maintained on a regular herpes immunization schedule.

ERICA LARSON

A researcher described an alternate testing procedure for detecting IR in horses, especially those animals with equine metabolic syndrome.

Rhinitis in Respiratory Cases

A common assumption among veterinarians is animals with a viral respiratory infection have either influenza or rhinopneumonitis (a respiratory condition, mostly of young horses, caused by EHV), said the University of Guelph's Andres Diaz-Mendez, Med Vet, MSc. However, another viral infection—equine rhinitis—is commonly responsible for respiratory outbreaks

Diaz-Mendez described clinical signs of equine rhinitis A virus (ERAV), which mimic those of flu or EHV: nasal discharge ranging from serous (clear, runny) to mucopurulent (thick, greenish-white), fever, and cough. In one study of nasal swabs from horses with acute respiratory disease, scientists found flu prevalence was 56%, ERAV was 42%, and 24% were infected with both viruses. He said that ERAV affects the upper and lower airways with moderate inflammation, exacerbating inflammatory airway disease (IAD) and recurrent airway obstruction (heaves).

Genome sequencing of ERAV has shown that any changes since it was first isolated in 1962 have been very minor; the currently circulating virus is 96% similar to that isolation. This is in contrast to equine influenza virus, which mutates frequently.

In Diaz-Mendez's study he induced experimental infection in a dozen 8- to 12-month-old healthy ponies by suppressing their immune systems with the corticosteroid dexamethasone three days prior to viral exposure. Within 24 hours of exposure, clinical signs began to appear as fever and increased wheezing lung sounds.

By Day 3, the ponies' submandibular (under the jaw) lymph nodes were enlarged and they had nasal discharge and tracheal mucus confirmed on endoscopy; the tracheal mucus persisted until Day 21. Lower airway samples revealed viral shedding Days 5 to 7. In the infected ponies an antibody response appeared by Day 7 that persisted to Day 21, along with airway inflammation lasting for three weeks. By Day 21, the lymph nodes were still enlarged but nasal discharge had abated.

The ERAV can impact the horse for three weeks. Diaz-Mendez noted that infection with ERAV might be considerably underestimated in the equine population and, thus, practitioners should consider it when assessing a case of acute respiratory viral infection. In addition, he pointed out that equine rhinitis virus might be associated with IAD and heaves episodes, among other respiratory conditions. At this time there is no ERAV vaccine available.

Diaz-Mendez continues to research ERAV and received one of three inaugural $25,000 Boehringer Ingelheim Vetmedica Equine Research Awards at the convention. In a study to be completed this year he'll examine equine airway epithelium response to ERAV infection and perform viral surveillance.

New 'Cancer Vaccine' for Horses

Cancer isn't diagnosed nearly as frequently in horses as it is in humans, but approximately 80% of all white or gray horses will develop melanomas by the time they are 15 years old. Currently there are few effective treatment options, partly because of melanomas' preferred location (near the tail, anus, groin, or salivary glands) and partly because they often aren't diagnosed early enough.

"One novel strategy for treating cancer in human medicine is the use of DNA vaccines that 'target' cancer cells," said Jeffrey Phillips, DVM, MS, PhD, Dipl. ACVIM, of Lincoln Memorial University's College of Veterinary and Comparative Medicine.

Melanocytic tumors (tumors arising from melanin-containing cells), including those diagnosed in horses, have a high level of "tyrosinase" molecules within their cells, which typically are not present in noncancerous cells. The DNA vaccine helps the immune system learn that these high levels of tyrosinase molecules are foreign, stimulating the immune system to identify and kill any cell containing elevated tyrosinase levels. In this respect the process is similar to other vaccines used in horses, such as those directed against bacteria (e.g., *Streptococcus equi*, which causes strangles) and viruses (e.g., West Nile virus, or WNV), in which the immune system is stimulated to attack molecules deemed "foreign."

In a small pilot study Phillips and colleagues used a commercially available canine DNA vaccine against tyrosinase in five apparently cancer-free horses (one

gray and four bays). None of the horses experienced any adverse reactions, and all horses developed an "appropriate," detectable, and long-lasting (likely more than six months) immune response—both humoral (antibody protection in the blood) and cell-mediated (activation of certain white blood cells as a response to a disease threat; thus activating both "sides" of the immune cascade)—to the vaccine.

"The DNA vaccine used in this study appears promising (based on safety and significant immune response against tyrosinase) in the treatment of equine melanomas," Phillips concluded. "Our initial results in horses with melanomas confirmed these findings and demonstrated tumor shrinkage, as was presented (previously) at AAEP. We are currently conducting field trials in horses with tumors."

New Insulin Resistance Test Method

An obese horse is often—though not always—an insulin-resistant (IR) one, and detection methods for insulin resistance can be tricky to time, not to mention labor-intensive. François R. Bertin, DVM, a resident at the Purdue University College of Veterinary Medicine described a new testing technique he has found useful for identifying insulin-resistant horses.

The pancreas' beta cells are responsible for secreting insulin to stimulate glucose uptake by glucose receptors. The glucose is moved into muscle cells where it is used immediately or stored as glycogen for later access. An IR horse has a decreased insulin sensitivity (as a result, higher amounts of insulin are released than normal in response to ingestion of starch and/or sugar) along with a decreased maximal response to this hormone. High levels of circulating insulin in the blood could put the horse at risk for developing laminitis.

The recommended insulin response test requires taking several blood samples over four hours. In a research trial Bertin used a two-step alternate procedure for testing 12 horses around 16 years of age with body condition scores ranging from 3-9 (5 being optimal). The horses were fed only free-choice hay and water. After measuring the baseline blood glucose of each horse, insulin was administered and another blood sample was taken 30 minutes later. Glucose readings were obtained stall-side with a hand-held glucometer, much like one used by human diabetics. If signs of hypoglycemia (sweating, shaking, or lying down) were noted following insulin treatment, Bertin recommended administering a dose of intravenous dextrose.

The conventional IR test and the two-step alternative test were performed on all of the horses. Bertin used the results of the conventional test to confirm whether the horse was considered insulin-resistant. He then compared the results of the two-step test with those of the conventional test to determine the former's accuracy.

He and his colleagues saw no significant differences between the insulin-resistant and insulin-sensitive horses at the baseline glucose measurement or in body condition score. Most of the blood glucose decrease occurred within 30 minutes of insulin treatment. Bertin reported that a decrease in glucose levels by 50% or more before 30 minutes indicated a horse was insulin-sensitive. With this test, all insulin-sensitive horses reached the 50% threshold of glucose drop within 30 minutes of insulin treatment. The IR horses, however, took longer than 30 minutes to reach this.

Bertin concluded that the test correlated well with the conventional test, it was repeatable, and he observed no adverse side effects. He noted, "This two-step insulin-response test had the same accuracy, sensitivity, and specificity as the complete conventional insulin-sensitivity test." The equine practitioner now has a useful and reliable low-cost IR test to use in the field, he concluded.

Bertin also noted that while overweight horses are more likely to be insulin-resistant than their slimmer counterparts, "it is important to say that there are obese healthy horses and insulin-resistant slim horses."

Seasonal Effects on Cushing's Test Results in Texas Horses

In horses with Cushing's disease, veterinarians often measure blood levels of adrenocorticotropin hormone (ACTH) to monitor treatment response. Seasonal variations in these levels—important for test result accuracy and treatment recommendations—have been confirmed in several geographic areas, with no seasonal changes reported in others. Ben Buchanan, DVM, Dipl. ACVIM, Dipl. ACVECC, and colleagues examined seasonal ACTH testing in Texas horses to establish norms for the region.

Buchanan, of Brazos Valley Equine Hospital in Navasota, reviewed that older horses are subject to developing benign tumors (adenomas) or enlargement of the pituitary gland, often referred to as Cushing's disease or pituitary pars intermedia dysfunction (PPID). Resulting decreases in dopamine production allow the pars intermedia to become overactive, which leads to an elevation in circulating ACTH. Alterations in the pituitary hormones put these horses at risk for developing laminitis, along with chronic infections and the inability to absorb nutrients optimally. Current Cushing's disease treatment includes pergolide, a dopamine agonist that mimics the effects of dopamine in PPID horses—this effect suppresses ACTH secretion. Buchanan noted that there is a known seasonal ACTH increase in all horses during late summer and fall, recognized as normal, likely due to the effect of changing hours of daylight on the pituitary gland.

The study consisted of 15 horses: Three had definitive PPID diagnoses; the other dozen were confirmed non-PPID horses. Fasting blood samples were obtained monthly for a year between 8 and10 a.m. and shipped to reliable reference labs for ACTH and insulin level analysis. Feed and pasture samples also were analyzed.

"We need to establish seasonal normal values or we should avoid testing in autumn months if we are only using a single value for year-round analysis," urged Buchanan.

In the study, the healthy horses had greater than 35 pg (picograms)/mL of ACTH in late summer and fall—a level usually associated with a PPID diagnosis. Such variations could lead to false positives in autumn months.

Buchanan noted that season did not significantly affect plasma insulin concentrations. However, there were differences throughout the year related to changes in pasture nonstructural carbohydrate content that might exert significant effects that could trigger pasture-associated laminitis. He commented that weather itself likely has more to do with pasture nonstructural carbohydrate content than overall seasonal influences and recommends that researchers conduct further studies to determine the role of pasture and how it impacts insulin resistance and PPID.

"The take-home message is that ACTH

concentrations in Texas are affected by season," Buchanan concluded. "Our research was consistent with others' findings in the U.S. and abroad."

Effects of Tramadol Use in Horses

Tramadol hydrocholoride is a medication physicians prescribe due to its opioid effects on the human central nervous system. Although it's not yet labeled for veterinary use, practitioners have used it in dogs and cats. Heather Knych, DVM, PhD, of the University of California, Davis, discussed a trial in which she and colleagues evaluated tramadol's pharmacokinetic (action in the body over a period of time) and behavioral effects.

"We are trying to find a drug that achieves analgesia with minimal side effects," Knych explained.

Generally, researchers determine what concentration of a drug in the plasma is therapeutic for the patient. Previous studies have failed to achieve in horses what are considered therapeutic tramadol plasma concentrations for humans. In this study the team evaluated three different dosage strengths administered to nine mature horses averaging 10 years of age. The animals received no medications for the two weeks prior to the study. Tramadol tablets, dissolved in water, were given by nasogastric tube. In a follow-up study a month later, the horses received three different dosage strengths of a compounded tramadol formulation intravenously.

"Oral administration," reported Knych, "led to a dose-dependent increase in the plasma concentration of tramadol." The half-life of the drug—meaning the amount of time it took for the drug concentration in the blood to reduce by half—ranged from two and a half to three hours.

Knych described the measurements used to evaluate the degree of the medication's behavioral effects: a) step counts; and b) chin-to-ground distance, which would likely be increased as a result of opiate-induced excitation. They also evaluated each horse's heart rate and rhythm for four to six hours following treatment.

The step count, or the number of steps the horse took over 10 minutes, did not change much over four hours for all three oral doses, although she noted that there was some variability between horses. Knych noted that the horses treated orally did not seem to be affected behaviorally with muscle fasciculations or tremors as was seen with intravenous administration.

The chin-to-ground distance did not significantly decrease over time at any dose, nor did the cardiac effects vary between dosages. Knych stressed, "There were no significant undesirable behavioral or pharmacokinetic effects at any dose."

> **"We are trying to find a drug that achieves analgesia (to address equine pain) with minimal side effects."**
>
> DR. HEATHER KNYCH

Concentrations capable of eliciting analgesia in humans were achieved in horses at the two higher oral doses; however, it is not yet known what the effective analgesic plasma concentration might be for horses. Based on efficacious plasma concentrations determined in humans, Knych said it might be necessary to administer tramadol two to three times per day at the higher doses for a horse to feel any analgesic effect based on the half-life of elimination.

Adverse Effects of Behavior-Modifying Drug Investigated

It might be tempting to initiate long-term sedation when confinement, stall rest, and tractability are necessary. John Baird, BVSc, PhD of the University of Guelph described the side effects that might occur following use of a long-acting anti-psychotic drug, fluphenazine decanoate.

This drug is used to treat humans with schizophrenia and is considered a performance enhancer by racing jurisdictions and show organizations. It's been known to cause a variety of adverse side effects, the most notable of which is dystonia, a condition characterized by abnormal involuntary muscle movements, said Baird.

Fluphenazine binds to dopamine receptors located in the extra-pyramidal system (part of the nervous system that controls movements), resulting in a blockage of the brain's dopamine pathways. This results in sustained muscle contractions, seen as peculiar postures and twisting of body parts. The drug also can elicit repetitive patterned movements, particularly of the neck, back, face, and tongue. Affected horses might also display akathisia—restlessness and a need to move, particularly the legs, coupled with anxiety or agitation.

Baird stressed that fluphenazine is highly potent and able to cross the blood-brain barrier to accumulate within the brain. When given to a pregnant mare, it is also able to enter the fetal circulation and the mare's milk. Horses that experience adverse side effects display the abnormal postures and severe depression almost to the point of somnolence (drowsiness), as well as the repetitive motions that at times could be accompanied by dangerous behavior. Many of these odd behaviors can be confused with other serious conditions, such as colic, rabies, EHV myeloencephalopathy, WNV encephalitis, or moldy corn poisoning. Baird remarked that there is no association between the severity of adverse signs and the plasma concentration of fluphenazine.

He showed case study videos depicting abnormal effects, such as rhythmic head tossing, pawing, sweating, or circling. Some of the cases he presented had received multiple doses of fluphenazine, while others had received only a single dose. While side effects might become apparent within 18-24 hours of injection, it's possible that they won't appear until weeks later.

To counteract these adverse physical and behavioral changes, veterinarians administer the antihistamine diphenhydrazine and/or the Parkinson's drug benztropine mesylate. In severe cases, horses might need a barbiturate (pentobarbital) so they don't injure themselves.

Aside from the the possible adverse effects, veterinarians and owners should remember that fluphenazine is a forbidden substance for horses competing in United States Equestrian Federation events, and it is banned in Fédération Equestrian International competitions, as well. There is at least a 90-day withdrawal time, meaning a horse might test positive up to 90 days after administration.

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