

New Modified-Live Equine Influenza Virus Vaccine: Safety and Efficacy Studies in Young Equids

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A new live attenuated intranasal equine influenza virus vaccine was effective in young equids in preventing clinical disease signs induced by wild-type equine-2 influenza virus strains of diverse origin, including both American and Eurasian lineages. The attenuated vaccine virus was safe and stable through serial pony-to-pony passages. Appendix: Tips for Intranasal Vaccination in Young Equids. Authors' addresses: Dept. of Veterinary Science, University of Kentucky, 108 Gluck Equine Research Center, Lexington, KY 40546-0099 (Chambers and Holland); Dept. of Veterinary Internal Medicine, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon SK S7N 5B4, Canada (Townsend, Cook, and Bogdan); Dept. of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, 2015 Linden Drive West, Madison, WI 53706 (Lunn and Hussey); Dept. of Molecular Genetics and Biochemistry, University of Pittsburgh, School of Medicine, E-1240 Biomedical Science Tower, Pittsburgh, PA 15261 (Whitaker-Dowling and Youngner); Heska Corporation, 1613 Prospect Parkway, Fort Collins, CO 80525 (Sebring, Penner, and Stiegler). © 1999 AAEP.

1. Introduction

Equine-2 influenza virus remains in circulation in most parts of the world including the United States. Recent studies of viral antigenic drift have identified two diverging lineages of equine-2 influenza¹: the American lineage, which is now widespread, and the Eurasian lineage, which has been only isolated once in the western hemisphere. Ideally, a new equine-2 influenza vaccine should protect horses against the latest virus strains and also against strains from both lineages. Also, for modified-live virus vac-

cines, the modification phenotype must be stable; i.e. an attenuated virus should not revert to virulence. The issues of heterologous protection and attenuation stability were tested in experiments using a novel modified-live equine-2 influenza virus (MLV) vaccine.

2. Materials and Methods

MLV vaccine was a cold-adapted variant of the Kentucky/91 strain of equine-2 influenza virus. Two separate heterologous challenge experiments were

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performed, one for each of the challenge viruses of the American lineage and Eurasian lineage. MLV vaccine was reconstituted from the lyophilized state and administered by a novel intranasal delivery device, in a single dose in one nostril. For the American lineage trial, the MLV vaccination group and seronegative control group each consisted of four weanling ponies. For the Eurasian lineage trial, MLV vaccination and control groups each consisted of 10 yearling horses. For both, challenge was done approximately 1 month following vaccination. Heterologous challenge viruses were administered by aerosol inhalation. Clinical signs were observed daily and converted to a clinical score using a standardized system.

To assess vaccine virus safety and stability through serial passage, 16 seronegative weanling ponies were used. Initially, five ponies were vaccinated intranasally using the MLV vaccine. Nasal swab fluids were collected and their virus titers determined, and fluids with the highest titers were pooled and administered intranasally to two other ponies. This process was repeated through two additional groups of two ponies and a final group of five ponies. Clinical signs were recorded from each group and converted to clinical incidence scores using the previously mentioned standardized system.

3. Results

Pretesting of the challenge viruses demonstrated induction of typical clinical signs for influenza.² Vaccination of seronegative ponies with the MLV vaccine did not induce clinical signs or adverse reactions. In each of the independent challenges using American or Eurasian lineage challenge viruses, the vaccinates exhibited significantly reduced clinical signs of disease (rectal temperatures and clinical sign scores for coughing and nasal discharge) relative to controls. Furthermore, both the frequency of horses shedding virus in the days after challenge and the number of days of shedding were reduced in vaccinates. Therefore, the vaccine clearly demonstrated clinical protection in two independent challenge models of the disease using recent and relevant influenza strains.

In the serial vaccine virus passage study, none of the 16 ponies exhibited clinical signs related to influenza disease. Most ponies seroconverted to equine-2 influenza, and all but one shed detectable virus at least once. Virus isolates from the final group were tested *in vitro*, and all retained the attenuation phenotype in those tests.

4. Discussion

Our results demonstrate that the new MLV vaccine, administered intranasally in a single dose to young seronegative equids, was effective in protecting them from clinical disease caused by a recent equine-2 influenza virus isolate from the United States and also an isolate of the Eurasian lineage. These strains were not components of the vaccine. The

American strain is the latest strain to be characterized in the United States. Therefore, the MLV vaccine is expected to be effective against the equine-2 influenza strains now in circulation. Also, our results demonstrate that through five consecutive direct pony-to-pony passages, the MLV vaccine retained its attenuation based on clinical evaluation and *in vitro* testing. Thus, the MLV vaccine is expected to be stable, safe, and effective in reducing influenza disease in young horses.

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The following useful tips for administration of intranasal vaccines to horses were developed by Robert E. Holland, DVM, PhD, and Thomas M. Chambers, PhD, for use with intranasal vaccines in general.

5. Appendix

A. Procedure for Intranasal Vaccine Administration

Prepare the vaccine following the package instructions from its manufacturer and using the applicator provided. Be certain to note the specific loading instructions, as some manufacturers may direct the vaccine be drawn up differently. An extremely helpful tip is to use a Luer lock syringe for the administration of these vaccines. Non-Luer-Lok syringes can come loose and be clumsy and dangerous.

Run your hand over the horse's nose a couple of times to see how sensitive the horse's nares are to manipulation. Agitation at this stage can be an excellent indicator that the horse will need some further restraint, such as a humane twitch.

Hold the vaccine syringe in your left hand, and stand on the left side of the horse. Hold the delivery device by the syringe, just below the Luer lock mechanism. Place your right hand on the horse's face just above the level of the nostrils. Place your right thumb just on top of the horse's left nostril opening. Use your thumb to keep the nostril wide while inserting the applicator. Use a smooth even motion to insert the applicator into the ventral nasal meatus.

Spray the vaccine using one steady motion of the syringe plunger, and not too fast. It should be given approximately over a 1-second time frame. Smoothly pull the device straight out of the horse's nose.

Hold the horse's head slightly higher than parallel to the ground for 30 seconds. This helps coat the mucosal surfaces and reduces the potential for some of the vaccine to drip out of the nose. Sometimes a few drops will fall out and that appears not to interfere with the effectiveness of the vaccine. Also, it is common to see a little residue of the vaccine solution still in the nasal applicator device. This is normal and does not interfere with the vaccine effectiveness if most of the vaccine was delivered correctly.

INFECTIOUS DISEASE

Following are some do's and don'ts to remember for intranasal vaccine administration.

B. Do's.

Do vaccinate the horse within a stall or small pen. This is helpful for control in case it acts up. The horse should have a lead rope or lead shank, with a handler.

Do store the vaccines refrigerated at 4°C (40°F) unless otherwise recommended by the manufacturer. Most intranasal vaccines are modified-live organisms that are easily damaged by heat. Mix the intranasal vaccine at the stall of the horse, preferably within less than 1 hour of use.

Do use a Luer-locking syringe if a syringe is needed, and make sure that the applicator is securely attached. The Luer-lock syringe is important as a safeguard to prevent the delivery tip from shooting off under pressure inside the horse's nose.

Do use a separate syringe for each horse to be vaccinated. Use a syringe size of 3 or 5 cc depending upon the volume of vaccine to be delivered.

Do wash your hands after you have given the intranasal vaccine and before giving any other medications (especially injections).

Do dispose of the vaccine vials, syringes, and nasal applicators per the recommendations made by the individual vaccine manufacturers.

C. Don'ts

Do not pre-mix the night before an appointment to vaccinate. The modified-live organisms in the vaccine will quickly begin to deteriorate and their numbers will decrease over time.

Do not insert the applicator tip into the dorsomedial blind pocket inside the horse's nostril. If you do hit this blind pocket, pull the nasal applicator back out and start over, repositioning the applicator in the correct position in the ventral nasal meatus.

Do not wiggle the applicator side-to-side within the horse's nose. Horses are very sensitive to movement inside their nose. Use your right thumb, which has been holding the nostril open, to keep the syringe and applicator in place while your left hand squeezes the plunger.

Do not put the whole syringe inside the nose. Only the applicator tip including the Luer-lock needs to be inside the nostril.

Do not let the halter nose band put pressure on the left side of the face, which could occlude the flow of the nasal applicator into the nasal passageway.

Do not push the vaccine through the syringe too quickly. If you do, much of the dose will fly right through into the pharynx, and then the horse may swallow it.

Do not reuse any of these materials. Do not try to clean the syringes or applicator tips with bleach or other agents for reuse, as residue of the agent will inactivate any modified-live vaccine used later.

References

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